SAMPLE

Matrix: urine

Sample preparation: 500 μL Urine + N-ethylnordiazepam + chlorpheniramine + 100 μL buffer, centrifuge at 11000 g for 30 s, inject a 500 μL aliquot onto column A with mobile phase A, after 0.6 min backflush column A with mobile phase A to waste for 1.6 min, elute column A with 250 μL mobile phase B, with 200 μL mobile phase C, and with 1.15 mL mobile phase D. Elute column A to waste until drugs start to emerge then elute onto column B. Elute column B to waste until drugs started to emerge, then elute onto column C. When all the drugs have emerged from column B remove it from the circuit, elute column C with mobile phase D, monitor the effluent from column C. Flush column A with 7 mL mobile phase E, with mobile phase D, and mobile phase A. Flush column B with 5 mL mobile phase E then with mobile phase D. (Buffer was 6 M ammonium acetate adjusted to pH 8.0 with 2 M KOH.)

HPLC VARIABLES

Column: A 10 × 2.1 12-20 μm PRP-1 spherical poly(styrene-divinylbenzene) (Hamilton); B 10 × 3.2 11 μm Aminex A-28 (Bio-Rad); C 25 × 3.2 5 μm C8 (Phenomenex) + 150 × 4.6 5 μm silica (Macherey-Nagel)

Mobile phase: A 0.1% pH 8.0 potassium borate buffer; B 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid; C MeCN:buffer 40:60 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.); D MeCN:buffer 33:67 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.); E MeCN:buffer 70:30 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.)

Column temperature: ambient (column A), 40 (columns B and C)

Flow rate: A 5; B-E 1

Injection volume: 500

Detector: UV 210, UV 235

CHROMATOGRAM

Retention time: k' 0.7

Internal standard: N-ethylnordiazepam (k' 2.1), chlorpheniramine (k' 5.9)

Limit of detection: 300 ng/mL

OTHER SUBSTANCES

Extracted: oxazepam, phenobarbital, nordiazepam, diazepam, phenylpropanolamine, phentermine, amphetamine, phenmetrazine, lidocaine, ephedrine, pentazocine, methamphetamine, desipramine, nortriptyline, diphenhydramine, methadone, imipramine, flurazepam, amitriptyline, morphine, codeine, hydromorphone, hydrocodone

Interfering: cotinine, benzoylecgonine, secobarbital

KEY WORDS

column-switching

REFERENCE

Binder,S.R.; Regalia,M.; Biaggi-McEachern,M.; Mazhar,M. Automated liquid chromatographic analysis of drugs in urine by on-line sample cleanup and isocratic multi-column separation, *J.Chromatogr.*, **1989**, *473*, 325–341.

SAMPLE

Matrix: urine

Sample preparation: Buffer urine to 4.9 by mixing with an equal volume of pH 4.9 200 mM sodium phosphate buffer. Inject a 40 μL aliquot onto column A with mobile phase A, after 3 min backflush the contents of column A onto column B with mobile phase B and start the gradient. At the end of the run re-equilibrate for 10 min.

HPLC VARIABLES

Column: A 20 × 4 5 μm Hypersil octadecylsilica ODS; B 200 × 4.6 5 μm Shiseido SG-120 polymer-based C18

Mobile phase: A water; B Gradient. MeCN:buffer from 7:93 to 15:85 over 3.5 min, to 50:50 over 8.5 min, maintain at 50:50 for 11 min (Buffer was 6.9 g $NaH_2PO_4.H_2O$ in 1 L water, pH adjusted to 3.1 with phosphoric acid.)

Flow rate: 1

Injection volume: 40

Detector: UV 270

CHROMATOGRAM

Retention time: 10.3

Limit of detection: 1000 ng/mL

OTHER SUBSTANCES

Extracted: acetazolamide, amiloride, bendroflumethiazide, benzthiazide, bumetanide, carbamazepine, chlorothiazide, chlorthalidone, clopamide, dichlorfenamide, ethacrynic acid, furosemide, hydrochlorothiazide, metyrapone, probenecid, spironolactone, triamterene, trichlormethiazide

KEY WORDS

column-switching; optimum detection wavelengths vary for each drug

REFERENCE

Saarinen,M.; Sirén,H.; Riekkola,M.-L. A column switching technique for the screening of diuretics in urine by high performance liquid chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 4063–4078.

SAMPLE

Matrix: urine

Sample preparation: 5 mL Urine + 50 μL 100 μg/mL 7-propyltheophylline in MeOH + 200 μL ammonium chloride buffer + 2 g NaCl, extract with 6 mL ethyl acetate by rocking at 40 movements/min for 20 min and centrifuging at 800 g for 5 min, repeat extraction, combine organic layers, evaporate to dryness at 40° under a stream of nitrogen. Reconstitute in 200 μL MeCN:water 15:85 and inject 20 μL aliquots. (Ammonium chloride buffer was 28 g ammonium chloride in 100 mL water with the pH adjusted to 9.5 with concentrated ammonia solution.)

HPLC VARIABLES

Column: 75 × 4.6 3 μm Ultrasphere ODS

Mobile phase: Gradient. MeCN:100 mM ammonium acetate adjusted to pH 3 with concentrated phosphoric acid. From 10:90 to 15:85 over 2 min to 55:45 over 3 min to 60:40 over 3 min. Kept at 60:40 for 1 min, decreased to 10:90 over 1 min and equilibrated at 10:90 for 2 min.

Flow rate: 1

Injection volume: 20

Detector: UV 270

CHROMATOGRAM

Retention time: 2.8

Internal standard: 7-propyltheophylline (4.5)

OTHER SUBSTANCES

Simultaneous: xipamide, bumetanide, acetazolamide, amiloride, bendroflumethiazide, buthiazide, benzthiazide, canrenone, chlorthalidone, clopamide, cyclothiazide, diclofenamide, ethacrynic acid, furosemide, hydrochlorothiazide, mesocarb, morazone, piretanide, polythiazide, probenecid, spironolactone, torsemide, triamterene

REFERENCE
Ventura,R.; Nadal,T.; Alcalde,P.; Pascual,J.A.; Segura,J. Fast screening method for diuretics, probenecid and other compounds of doping interest, *J.Chromatogr.A*, **1993**, *655*, 233–242.

SAMPLE
Matrix: urine
Sample preparation: Urine. Stabilize urine with 1 M pH 3.0 citric acid phosphate buffer. 50 μL Urine + 120 mg ammonium sulfate + 50 μL 1.2 mg/mL acetaminophen + 6 mL chloroform:isopropanol 95:5, shake gently for 20 min, centrifuge at 2000 g for 5 min. Remove 5 mL of the organic layer and evaporate it to dryness, reconstitute the residue in 250 μL 0.05% acetic acid, inject a 50 μL aliquot. Plasma. 500 μL Plasma + 75 μL 1 M HCl + 25 μL 500 μg/mL acetanilide + 120 mg ammonium sulfate + 6 mL chloroform: isopropanol 95:5, shake gently for 20 min, centrifuge at 2000 g for 5 min. Remove 5 mL of the organic layer and evaporate it to dryness, reconstitute the residue in 250 μL 0.05% acetic acid, inject a 30 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: Gradient. MeOH:0.25% acetic acid 7.5:92.5 for 10 min then to 21:79 over 20 min, maintain at 21:79 for 5 min, then to 60:40 over 5 min, return to 7.5:92.5 over 5 min, re-equilibrate for 15 min. (urine) Isocratic. MeOH:0.25% acetic acid 20:80. (plasma)
Flow rate: 1.2
Injection volume: 50
Detector: UV 280

CHROMATOGRAM
Internal standard: acetaminophen (urine), acetanilide (plasma)

OTHER SUBSTANCES
Extracted: metabolites

REFERENCE
Rost,K.L.; Roots,I. Accelerated caffeine metabolism after omeprazole treatment is indicated by urinary metabolite ratios: Coincidence with plasma clearance and breath test, *Clin.Pharmacol.Ther.*, **1994**, *55*, 402–411.

SAMPLE
Matrix: urine
Sample preparation: Dilute urine 10-fold with 5 μg/mL β-hydroxyethyltheophylline in water, mix, centrifuge at 14000 rpm for 2 min, inject a 25 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 40 × 2.5 10 μm Lichrosorb RP-2
Column: 150 × 4.6 5 μm Ultrasphere-ODS
Mobile phase: MeCN:THF:10 mM sodium acetate 3:0.1:96.9 containing 5 mM tetrabutylammonium hydrogen sulfate, pH 4.7
Flow rate: 1.5
Injection volume: 25
Detector: UV 280

CHROMATOGRAM
Retention time: 18
Internal standard: β-hydroxyethyltheophylline (11)
Limit of detection: 1 μg/mL

OTHER SUBSTANCES
Extracted: metabolites, 1,3-dimethyluric acid, 1-methyluric acid, 3-methylxanthine, theophylline

REFERENCE
Tajerzadeh,H.; Dadashzadeh,S. An isocratic high-performance liquid chromatographic system for simultaneous determination of theophylline and its major metabolites in human urine, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 1507–1512.

SAMPLE
Matrix: urine
Sample preparation: Condition a 700 mg Extrelut-1 diatomaceous earth glass SPE cartridge with 6 mL dichloromethane, let stand for 1 day. 200 μL Urine + 200 μL 10 μg/mL N-ethylnornicotine in water + 600 μL 500 mM NaOH, add to the SPE cartridge, let stand for 10 min, elute with 5 mL dichloromethane:isopropanol 90:10. Add the eluate to 100 μL 25 mM HCl in MeOH and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL water, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm LC8DB (Supelchem)
Mobile phase: MeCN:water 9:80 containing 5 mL/L triethylamine, 670 mg/L sodium heptanesulfonate, 34 mM K_2HPO_4, and 34 mM citric acid, pH 4.4.
Flow rate: 1.6
Detector: UV 254

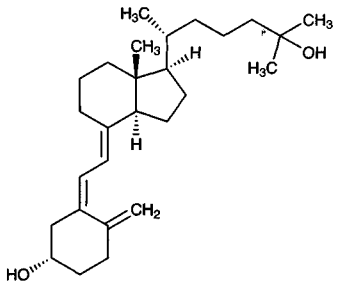
CHROMATOGRAM
Retention time: 6
Internal standard: N-ethylnornicotine (8.5)

OTHER SUBSTANCES
Extracted: metabolites, cotinine, nicotine

KEY WORDS
SPE

REFERENCE
Zuccaro,P.; Altieri,I.; Rosa,M.; Passa,A.R.; Pichini,S.; Pacifici,R. Solid-phase extraction of nicotine and its metabolites for high-performance liquid chromatographic determination in urine, *J.Chromatogr.B*, **1995**, *668*, 187–188.

Calcifediol



Molecular formula: $C_{27}H_{44}O_2$
Molecular weight: 400.65
CAS Registry No.: 19356-17-3, 63283-36-3 (monohydrate)
Merck Index: 1677
Lednicer No.: 3 101

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL MeOH, allow to stand for 30 min, add 10 mL hexane, shake for 10 min, centrifuge at 2000 rpm for 10 min. Remove 9 mL of the organic phase and evaporate it under nitrogen at 55°, reconstitute with 300 μL mobile phase A, inject a 250 μL aliquot onto column A with mobile phase A. Collect the material corresponding to the retention time of calcifediol, evaporate it to dryness, reconstitute in mobile phase B, inject an aliquot on column B with mobile phase B.

HPLC VARIABLES

Column: A Brownlee guard column + 250 × 4.6 5 μm Li-Chrosorb SI-100; B 150 × 4.6 Ultrasphere Octyl C8
Mobile phase: A Hexane:EtOH 90:10; B MeCN:water 80:20
Flow rate: A 2; B 1.5
Injection volume: 250
Detector: UV 254

CHROMATOGRAM

Retention time: 3 (A), 4 (B)
Limit of quantitation: 2 ng/mL

KEY WORDS

plasma; normal phase

REFERENCE

Loo,J.C.; Brien,R. Analysis of 25-hydroxy vitamin D3 in plasma by high-performance liquid chromatography, *Res.Commun.Chem.Pathol.Pharmacol.*, **1983**, *41*, 139–148.

SAMPLE

Matrix: blood
Sample preparation: Condition a C18 Sep Pak SPE cartridge with 5 mL hexane, 5 mL chloroform, 5 mL MeOH, and 5 mL water. 3 mL Serum + 3 mL MeCN, vortex, centrifuge at 1500 g for 10 min. Remove the supernatant and add it to 1.5 mL 400 mM pH 10.6 K_2HPO_4, add the mixture to the SPE cartridge, wash with 5 mL water, wash with 3 mL MeOH:water 70:30, elute with 4 mL MeCN, evaporate the eluate to dryness under a stream of nitrogen, reconstitute in mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 150 × 4.5 3 μm Spherisorb
Mobile phase: MeOH:isopropanol:hexane 2:5:93
Flow rate: 1.5
Detector: UV 254 or radioreceptor assay

CHROMATOGRAM

Retention time: 2.5

OTHER SUBSTANCES
Extracted: calcitriol, 24,25-dihydroxyvitamin D

KEY WORDS
serum; SPE; normal phase

REFERENCE
Saggese,G.; Bertelloni,S.; Baroncelli,G.I. Dosaggio radiorecettoriale dei metaboliti della vitamina D dopo cromatografia liquida ad alta risoluzione con fasi stazionarie ultrafini, *Giorn.It.Chim.Clin.*, **1986**, *11*, 177–182.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve compound in 30 μL pyridine, add 25 μL N,O-bis-(trimethylsilyl)trifluoroacetamide containing 1% trimethylsilyl chloride, heat at 55° for 45 min, evaporate to dryness under a stream of nitrogen, reconstitute with 50 μL hexane, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.5 microparticulate silica
Mobile phase: Hexane:ethyl acetate 99.85:0.15
Flow rate: 2
Detector: UV 254

CHROMATOGRAM
Retention time: 7.5

KEY WORDS
derivatization; normal phase

REFERENCE
Wichmann,J.; Schnoes,H.K.; DeLuca,H.F. Isolation of identification of 24(R)-hydroxyvitamin D3 from chicks give large doses of vitamin D3, *Biochem.*, **1981**, *20*, 2350–2353.

SAMPLE
Matrix: solutions
Sample preparation: Evaporate solution of calcifediol in EtOH, add 1 mL 7.2 μM DMEQ-TAD in dichloromethane, stir at room temperature for 30 min, add EtOH, evaporate, dissolve residue in MeOH, inject an aliquot. (Dichloromethane should be MeOH free. Wash with concentrated sulfuric acid, water, 5% sodium carbonate, water, dry over calcium chloride, and distil from calcium hydride. DMEQ-TAD was 4-[2-(6,7-dimethoxy-4-methyl-3-oxo-3,4-dihydroquinoxalinyl)ethyl]-1,2,4-triazoline-3,5-dione. Synthesis is as follows. Stir 483 g veratrole in 1.45 L acetic acid at 15° for 1 h, add 683 g concentrated nitric acid (d 1.05) over 1 h (maintain the temperature below 40° by cooling and regulating the rate of addition of the nitric acid). Continue stirring and add 2.127 L fuming nitric acid (d 1.50) over 1 h while maintaining the temperature below 30°, let stand for 2 h, pour into a large volume of cold water, filter, wash the solid with water until the washings are neutral, recrystallize from EtOH to give 4,5-dinitroveratrole (mp 129.5-130.5°) (J. Am. Chem. Soc. 1946, 68, 1536). Shake a solution of 910 mg 4,5-dinitroveratrole in 80 mL EtOH with 89 mg platinum(IV) oxide under an atmosphere of hydrogen until the theoretical amount of hydrogen (540 mL) is absorbed, filter under nitrogen into a flask containing 580 mg 2-ketoglutaric acid, reflux this mixture for 1.5 h, cool, collect the precipitate, recrystallize from EtOH to obtain 6,7-dimethoxy-3-oxo-3,4-dihydroquinoxaline-2-propionic acid as a crystalline solid (mp 250-252°). Add a solution of 606 mg 6,7-dimethoxy-3-oxo-3,4-dihydroquinoxaline-2-propionic acid in 20 mL DMF under nitrogen to a suspension of 176 mg NaH in 3 mL DMF stirred at 0°, stir for 30 min, add 455 μL methyl iodide, stir at 0° for 1.5 h, pour into ice-water, stir at room temperature for 30 min, acidify

with 500 mM HCl, collect the precipitate, recrystallize from chloroform/MeOH to obtain 6,7-dimethoxy-4-methyl-3-oxo-3,4-dihydroquinoxaline-2-propionic acid (mp 239-241°). Add 360 μL triethylamine at room temperature to 500 mg 6,7-dimethoxy-4-methyl-3-oxo-3,4-dihydroquinoxaline-2-propionic acid in 50 mL DMF, add 550 μL diphenylphosphoryl azide, stir at room temperature for 2.5 h, evaporate to dryness under reduced pressure, dissolve the residue in 20 mL benzene (Caution! Benzene is a carcinogen!), reflux for 1 h, cool to room temperature, add 178 mg ethyl carbazate, reflux for 30 min, evaporate, chromatograph on 90 g silica gel, elute with chloroform to remove a by-product then with chloroform:MeOH 96:4 to obtain 1-ethoxycarbonyl-4-[2-(6,7-dimethoxy-4-methyl-3-oxo-3,4-dihydroquinoxalinyl)ethyl]semicarbazide. Reflux a suspension of 272 mg 1-ethoxycarbonyl-4-[2-(6,7-dimethoxy-4-methyl-3-oxo-3,4-dihydroquinoxalinyl)ethyl]semicarbazide and 190 mg potassium carbonate in 20 mL EtOH for 6 h, evaporate the solvent, dissolve the residue in 30 mL water, acidify with 2 M HCl, extract with chloroform:MeOH 90:10, dry over anhydrous sodium sulfate, evaporate, recrystallize from MeOH/chloroform to obtain 4-[2-(6,7-dimethoxy-4-methyl-3-oxo-3,4-dihydroquinoxalinyl)ethyl]-1,2,4-triazolidine-3,5-dione as pale yellow prisms (mp 250-253°). Add 10 mg iodobenzene diacetate to a stirred suspension of 8.6 mg 4-[2-(6,7-dimethoxy-4-methyl-3-oxo-3,4-dihydroquinoxalinyl)ethyl]-1,2,4-triazolidine-3,5-dione in 1.5 mL MeOH-free dichloromethane, stir at room temperature for 3.5 h, filter, store the filtrate at -20° overnight, filter under argon to obtain 4-[2-(6,7-dimethoxy-4-methyl-3-oxo-3,4-dihydroquinoxalinyl)ethyl]-1,2,4-triazoline-3,5-dione as red needles (mp 200-202° d).)

HPLC VARIABLES

Column: 250 × 4 LiChrospher RP-18(e)
Mobile phase: Gradient. MeOH:water from 60:40 to 80:20 over 40 min
Column temperature: 35
Flow rate: 1
Injection volume: 10
Detector: F ex 370 em 440

CHROMATOGRAM

Retention time: 33 and 36 (C6 epimers)

OTHER SUBSTANCES

Simultaneous: calcitriol

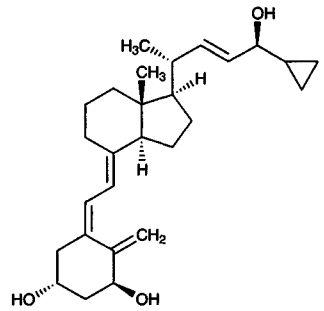
KEY WORDS

derivatization

REFERENCE

Shimizu,M.; Kamachi,S.; Nishii,Y.; Yamada,S. Synthesis of a reagent for fluorescence-labeling of vitamin D and its use in assaying vitamin D metabolites, *Anal.Biochem.*, **1991**, *194*, 77–81.

Calcipotriene



Molecular formula: $C_{27}H_{40}O_3$
Molecular weight: 412.61
CAS Registry No.: 112965-21-6
Merck Index: 1679
Lednicer No.: 5 60

SAMPLE

Matrix: cell cultures
Sample preparation: Extract cell cultures (Can. J. Biochem. 1957, 37, 911). Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in mobile phase, inject a 200 μL aliquot.

HPLC VARIABLES

Column: 80 × 6.2 Zorbax-SIL
Mobile phase: Hexane:isopropanol:MeOH 91:7:2
Flow rate: 1
Injection volume: 200
Detector: UV 265

CHROMATOGRAM

Retention time: 12

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

normal phase

REFERENCE

Masuda,S.; Strugnell,S.; Calverley,M.J.; Makin,H.L.J.; Kremer,R.; Jones,G. *In vitro* metabolism of the anti-psoriatic vitamin D analog, calcipotriol, in two cultured human keratinocyte models, *J.Biol.Chem.*, **1994**, *269*, 4794–4803.

SAMPLE

Matrix: cell suspensions
Sample preparation: 1.25 mL Liver homogenate + 1.25 mL MeCN, centrifuge, remove supernatant and dilute it with 2.5 mL water, pass through a Varian C8 AASP SPE cartridge, purge with 250 μL MeOH:water 10:90, elute contents of SPE cartridge onto HPLC column.

HPLC VARIABLES

Column: 125 × 4 5 μm LiChrospher RP 18
Mobile phase: Gradient. MeOH:water from 70:30 to 95:5 over 15 min
Flow rate: 2
Detector: UV 264

CHROMATOGRAM

Retention time: 9

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

rat; liver; human; SPE; pig

REFERENCE

Sorensen,H.; Binderup,L.; Calverley,M.J.; Hoffmeyer,L.; Andersen,N.R. *In vitro* metabolism of calcipotriol (MC 903), a vitamin D analogue, *Biochem.Pharmacol.*, **1990**, *39*, 391–393.

Calcitonin

Molecular formula: $C_{151}H_{226}N_{40}O_5S_3$
Molecular weight: 2777.9
CAS Registry No.: 9007-12-9, 47931-85-1 (salmon), 21215-62-3 (human)
Merck Index: 1680

SAMPLE

Matrix: blood
Sample preparation: Add 1 mL rat plasma and 100 ng rat calcitonin to 1 mL polypropylene column containing immobilized antibody column (preparation details in paper), wash with three 5 mL portions of water, elute with 5 mL MeOH. Evaporate the eluate to dryness under reduced pressure, reconstitute with MeOH:water:trifluoroacetic acid 50:50:1, inject a 5 μL aliquot.

HPLC VARIABLES

Column: 150 × 0.32 C18 (LC Packing, Zurich)
Mobile phase: Gradient. A was 0.1% trifluoroacetic acid in water. B was MeCN:water 60:40 containing 0.1% trifluoroacetic acid. A:B from 100:0 to 0:100 over 10 min.
Flow rate: 0.003
Injection volume: 5
Detector: LC-MS, Finnigan MAT TSQ-700, Analytica model electrospray ionization (ESI) source, manifold 70°, voltage -3.5 kV, drying gas for ESI nitrogen 68 kPA at 80°, gas sheath nitrogen 102 kPa, liquid sheath 2-methoxyethanol at 2 μL/min, m/z 1140 ± 2 (human), m/z 1132 ± 2 rat

CHROMATOGRAM

Retention time: 15.3
Internal standard: rat calcitonin
Limit of quantitation: 10 ng/mL

KEY WORDS

human; rat; plasma

REFERENCE

Kobayashi,N.; Kanai,M.; Seta,K.; Nakamura,K.-i. Quantitative analysis of synthetic human calcitonin by liquid chromatography-mass spectrometry, *J.Chromatogr.B*, **1995**, *672*, 17–23.

SAMPLE

Matrix: blood, tissue
Sample preparation: Homogenize (glass/PTFE homogenizer) kidney in 100 mM pH 7.4 Tris-HCl. Dilute plasma and tissue homogenates 10-fold with 0.1% trifluoroacetic acid in water, inject a 100 μL aliquot on to column a and elute to waste with mobile phase A, after 5 min backflush the contents of column A on to column B with mobile phase B and start the gradient, after 25 min re-equilibrate column A with mobile phase A and column B with mobile phase B (at initial conditions) for 5 min.

HPLC VARIABLES

Column: A 20 × 3.9 25-40 μm LiChroprep RP-8; B 10 × 4 Nova-Pak C8 + 250 × 4.6 5 μm W-Porex 5 C18 (Phenomenex)
Mobile phase: A 0.1% trifluoroacetic acid in water; B Gradient. A was 0.1% trifluoroacetic acid in water. B was 0.1% trifluoroacetic acid in MeCN. A:B from 75:25 to 45:55 over 20 min, to 0:100 over 5 min.
Flow rate: A 0.5; B 1.2
Injection volume: 100

Detector: radioactivity

CHROMATOGRAM
Retention time: 12
Limit of detection: 2.5 pg/mL

OTHER SUBSTANCES
Extracted: degradation products

KEY WORDS
salmon; ^{125}I labeled; rat; plasma; kidney; column-switching

REFERENCE
Lee,H.S.; Lee,J.S.; Lee,H.; Jung,Y.S.; DeLuca,P.P.; Lee,K.C. Reversed-phase high-performance liquid chromatography of salmon calcitonin and its degradation products in biological samples using column switching and flow-through radioisotope detection, *J.Chromatogr.B*, **1995**, *673*, 136–141.

SAMPLE
Matrix: formulations
Sample preparation: Polymeric beads. Dissolve 100 mg dried beads in 3 mL 20 mM pH 3 glycine/HCl buffer containing 150 mM NaCl, let stand at 4° overnight, heat up to 50° for 10 min. After precipitation of the polymer, inject an aliquot of the supernatant. Release experiment. Place polymeric beads in 10 mL pH 7.4 isotonic PBS at 37° or in 10 mL 10 mM pH 4.5 acetate buffer containing 150 mM NaCl at 15°. At different times, collect 100 μL release medium and replace by the same volume of buffer. Inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Vydac C4
Mobile phase: Gradient. A was MeCN:water 10:90 containing 0.1% trifluoroacetic acid. B was MeCN:water 60:40 containing 0.095% trifluoroacetic acid. A:B from 60:40 to 40:60 over 15 min
Flow rate: 1
Injection volume: 200
Detector: UV 214

CHROMATOGRAM
Retention time: 10

KEY WORDS
beads; stability-indicating

REFERENCE
Serres,A.; Baudys,M.; Kim,S.W. Temperature and pH-sensitive polymers for human calcitonin delivery, *Pharm.Res.*, **1996**, *13*, 196–201.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 150 × 4.6 5 μm M-C4 (pore diameter 60 Å) (ES Industries)
Mobile phase: Gradient. A was 10 mM ammonium acetate containing 0.1% trifluoroacetic acid. B was MeCN:20 mM ammonium acetate 50:50 containing 0.1% trifluoroacetic acid. A:B 45:5 for 5 min, to 40:60 over 35 min, maintain at 40:60 over 5 min.
Flow rate: 2
Injection volume: 200
Detector: UV 214

CHROMATOGRAM
Retention time: 30

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
salmon

REFERENCE
Lee,I.H.; Pollack,S.; Hsu,S.H.; Miksic,J.R. Influence of the mobile phase on salmon calcitonin analysis by reversed-phase liquid chromatography, *J.Chromatogr.Sci.*, **1991**, *29*, 136–140.

SAMPLE
Matrix: solutions
Sample preparation: Make up a solution in buffer, inject a 100 μL aliquot. (Buffer was 2 mg/mL sodium acetate and 5 mg/mL NaCl adjusted to pH 4.2 with glacial acetic acid.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm M-C4 (pore diameter 60 Å) (ES Industries)
Mobile phase: Gradient. MeCN:0.1% trifluoroacetic acid from 27.5:72.5 to 30:70 over 15 min
Flow rate: 2
Injection volume: 100
Detector: UV 214

CHROMATOGRAM
Retention time: 3 (human), 7.5 (salmon), 9 (eel), 13.5 (pig)

KEY WORDS
salmon; human; eel; pig

REFERENCE
Lee,I.H.; Pollack,S.; Hsu,S.H.; Miksic,J.R. Influence of the mobile phase on salmon calcitonin analysis by reversed-phase liquid chromatography, *J.Chromatogr.Sci.*, **1991**, *29*, 136–140.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 TSKgel ODS-120T
Mobile phase: Gradient. A was MeOH:water 20:80 containing 0.05% trifluoroacetic acid. B was MeOH:water 50:50 containing 0.05% trifluoroacetic acid. A:B from 100:0 to 0:100 over 1 h.
Flow rate: 1
Detector: UV 220

CHROMATOGRAM
Retention time: 26

OTHER SUBSTANCES
Simultaneous: angiotensin I, angiotensin II, α-endorphin, β-endorphin, gonadorelin (LH-RH), protirelin (TRH), somatostatin

KEY WORDS
human

REFERENCE

Varian Catalog, **1993**, p. 182.

SAMPLE

Matrix: solutions

Sample preparation: 5 μL Calcitonin in MeCN:0.2% aqueous phosphoric acid containing 100 mM sodium perchlorate 30:70 + 20 μL 250 mM pH 8.5 borate buffer containing 2.5 mM sodium dodecyl sulfate + 2.5 μL 30 mM 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole in MeCN, add 11 μL MeCN and 11.5 μL water, heat at 50 ° for 3 h, cool, add 5 μL 1 M HCl, add 40 μL MeCN, add 5 μL water, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm TSKgel ODS 80Tm (Tosoh)

Mobile phase: Gradient. A was MeCN containing 0.05% trifluoroacetic acid. B was water containing 0.05% trifluoroacetic acid. A:B 33:67 for 8 min, to 67:33 over 17 min.

Column temperature: 40

Flow rate: 1

Injection volume: 50

Detector: F ex 430 em 558

CHROMATOGRAM

Retention time: 26

Limit of detection: 71 pg

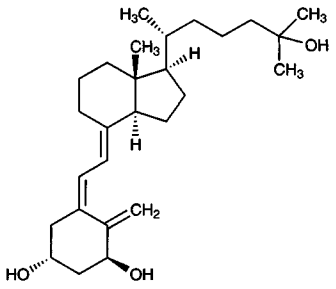
KEY WORDS

salmon; derivatization

REFERENCE

Fukuda,T.; Ishikawa,K.; Imai,K. Sensitive determination of salmon calcitonin, by means of pre-column derivatization, HPLC and fluorometric determination, *Biomed.Chromatogr.*, **1995**, *9*, 52–55.

Calcitriol



Molecular formula: $C_{27}H_{44}O_3$
Molecular weight: 416.64
CAS Registry No.: 32222-06-3
Merck Index: 1681
Lednicer No.: 3 103

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL MeOH, allow to stand for 30 min, add 10 mL hexane, shake for 10 min, centrifuge at 2000 rpm for 10 min. Remove 9 mL of the organic phase and evaporate it under nitrogen at 55°, reconstitute with 300 μL mobile phase, inject a 250 μL aliquot.

HPLC VARIABLES

Guard column: Brownlee guard column
Column: 250 × 4.6 5 μm Li-Chrosorb SI-100
Mobile phase: Hexane:EtOH 90:10
Flow rate: 2
Injection volume: 250
Detector: UV 254

CHROMATOGRAM

Retention time: 4

OTHER SUBSTANCES

Simultaneous: calcifediol

KEY WORDS

plasma; normal phase

REFERENCE

Loo,J.C.; Brien,R. Analysis of 25-hydroxy vitamin D3 in plasma by high-performance liquid chromatography, *Res.Commun.Chem.Pathol.Pharmacol.*, **1983**, *41*, 139–148.

SAMPLE

Matrix: blood
Sample preparation: Condition a C18 Sep Pak SPE cartridge with 5 mL hexane, 5 mL chloroform, 5 mL MeOH, and 5 mL water. 3 mL Serum + 3 mL MeCN, vortex, centrifuge at 1500 g for 10 min. Remove the supernatant and add it to 1.5 mL 400 mM pH 10.6 K_2HPO_4, add the mixture to the SPE cartridge, wash with 5 mL water, wash with 3 mL MeOH:water 70:30, elute with 4 mL MeCN, evaporate the eluate to dryness under a stream of nitrogen, reconstitute in mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 150 × 4.5 3 μm Spherisorb
Mobile phase: MeOH:isopropanol:hexane 2:5:93
Flow rate: 1.5
Detector: UV 254 or radioreceptor assay

CHROMATOGRAM

Retention time: 9.25

OTHER SUBSTANCES
Extracted: calcifediol (25-hydroxyvitamin D3), 24,25-dihydroxyvitamin D

KEY WORDS
serum; SPE; normal phase

REFERENCE
Saggese,G.; Bertelloni,S.; Baroncelli,G.I. Dosaggio radiorecettoriale dei metaboliti della vitamina D dopo cromatografia liquida ad alta risoluzione con fasi stazionarie ultrafini, *Giorn.It.Chim.Clin.*, **1986**, *11*, 177–182.

SAMPLE
Matrix: formulations
Sample preparation: Cut 25 capsules in half and add to 5 mL isooctane:isopropanol 90:10, filter through glass wool, wash capsule shells with three 5 mL aliquots of isooctane:isopropanol 90:10, combine filtrates and make up to 25 mL with isooctane:isopropanol 90:10, inject a 50 μL aliquot. Alternatively, inject directly 10 μL aliquots of oil in capsules.

HPLC VARIABLES
Column: Two 150 × 4.6 5 μm Ultrasphere-SI silica in series
Mobile phase: Hexane:THF:dichloromethane:isopropanol 72:12:12:4
Flow rate: 1
Injection volume: 10-50
Detector: UV 254

CHROMATOGRAM
Retention time: 16.3

OTHER SUBSTANCES
Simultaneous: 1α-hydroxycholecalciferol (alfacalcidol)

KEY WORDS
capsules; normal phase

REFERENCE
Flann,B.C.; Lodge,B.A. Validation of liquid chromatographic method for assay of calcitriol and alfacalcidol in capsule formulations, *J.Assoc.Off.Anal.Chem.*, **1986**, *69*, 1026–1030.

SAMPLE
Matrix: solutions
Sample preparation: Evaporate solution of calcifediol in EtOH, add 1 mL 7.2 μM DMEQ-TAD in dichloromethane, stir at room temperature for 30 min, add EtOH, evaporate, dissolve residue in MeOH, inject an aliquot. (Dichloromethane should be MeOH free. Wash with concentrated sulfuric acid, water, 5% sodium carbonate, water, dry over calcium chloride, and distil from calcium hydride. DMEQ-TAD was 4-[2-(6,7-dimethoxy-4-methyl-3-oxo-3,4-dihydroquinoxalinyl)ethyl]-1,2,4-triazoline-3,5-dione. Synthesis is as follows. Stir 483 g veratrole in 1.45 L acetic acid at 15° for 1 h, add 683 g concentrated nitric acid (d 1.05) over 1 h (maintain the temperature below 40° by cooling and regulating the rate of addition of the nitric acid). Continue stirring and add 2.127 L fuming nitric acid (d 1.50) over 1 h while maintaining the temperature below 30°, let stand for 2 h, pour into a large volume of cold water, filter, wash the solid with water until the washings are neutral, recrystallize from EtOH to give 4,5-dinitroveratrole (mp 129.5-130.5°) (J. Am. Chem. Soc. 1946, 68, 1536). Shake a solution of 910 mg 4,5-dinitroveratrole in 80 mL EtOH with 89 mg platinum(IV) oxide under an atmosphere of hydrogen until the theoretical amount of hydrogen (540 mL) is absorbed, filter under nitrogen into a flask containing 580 mg 2-ketoglutaric acid, reflux this mixture for 1.5 h, cool, collect the precipitate, recrystallize from EtOH to obtain 6,7-dimethoxy-3-oxo-3,4-dihydroquinoxaline-2-

propionic acid as a crystalline solid (mp 250-252°). Add a solution of 606 mg 6,7-dimethoxy-3-oxo-3,4-dihydroquinoxaline-2-propionic acid in 20 mL DMF under nitrogen to a suspension of 176 mg NaH in 3 mL DMF stirred at 0°, stir for 30 min, add 455 μL methyl iodide, stir at 0° for 1.5 h, pour into ice-water, stir at room temperature for 30 min, acidify with 500 mM HCl, collect the precipitate, recrystallize from chloroform/MeOH to obtain 6,7-dimethoxy-4-methyl-3-oxo-3,4-dihydroquinoxaline-2-propionic acid (mp 239-241°). Add 360 μL triethylamine at room temperature to 500 mg 6,7-dimethoxy-4-methyl-3-oxo-3,4-dihydroquinoxaline-2-propionic acid in 50 mL DMF, add 550 μL diphenylphosphoryl azide, stir at room temperature for 2.5 h, evaporate to dryness under reduced pressure, dissolve the residue in 20 mL benzene (Caution! Benzene is a carcinogen!), reflux for 1 h, cool to room temperature, add 178 mg ethyl carbazate, reflux for 30 min, evaporate, chromatograph on 90 g silica gel, elute with chloroform to remove a by-product then with chloroform:MeOH 96:4 to obtain 1-ethoxycarbonyl-4-[2-(6,7-dimethoxy-4-methyl-3-oxo-3,4-dihydroquinoxalinyl)]ethylsemicarbazide. Reflux a suspension of 272 mg 1-ethoxycarbonyl-4-[2-(6,7-dimethoxy-4-methyl-3-oxo-3,4-dihydroquinoxalinyl)ethyl]semicarbazide and 190 mg potassium carbonate in 20 mL EtOH for 6 h, evaporate the solvent, dissolve the residue in 30 mL water, acidify with 2 M HCl, extract with chloroform:MeOH 90:10, dry over anhydrous sodium sulfate, evaporate, recrystallize from MeOH/chloroform to obtain 4-[2-(6,7-dimethoxy-4-methyl-3-oxo-3,4-dihydroquinoxalinyl)ethyl]-1,2,4-triazolidine-3,5-dione as pale yellow prisms (mp 250-253°). Add 10 mg iodobenzene diacetate to a stirred suspension of 8.6 mg 4-[2-(6,7-dimethoxy-4-methyl-3-oxo-3,4-dihydroquinoxalinyl)ethyl]-1,2,4-triazolidine-3,5-dione in 1.5 mL MeOH-free dichloromethane, stir at room temperature for 3.5 h, filter, store the filtrate at -20° overnight, filter under argon to obtain 4-[2-(6,7-dimethoxy-4-methyl-3-oxo-3,4-dihydroquinoxalinyl)ethyl]-1,2,4-triazoline-3,5-dione as red needles (mp 200-202° d).)

HPLC VARIABLES

Column: 250 × 4 LiChrospher RP-18(e)
Mobile phase: Gradient. MeOH:water from 60:40 to 80:20 over 40 min
Column temperature: 35
Flow rate: 1
Injection volume: 10
Detector: F ex 370 em 440

CHROMATOGRAM

Retention time: 27 and 30 (C6 epimers)

OTHER SUBSTANCES

Simultaneous: calcifediol

KEY WORDS

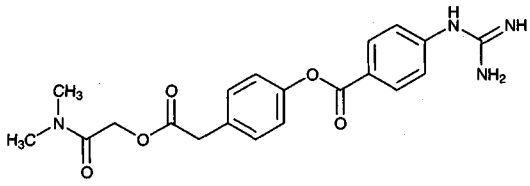
derivatization

REFERENCE

Shimizu,M.; Kamachi,S.; Nishii,Y.; Yamada,S. Synthesis of a reagent for fluorescence-labeling of vitamin D and its use in assaying vitamin D metabolites, *Anal.Biochem.*, **1991**, *194*, 77–81.

Camostat

Molecular formula: $C_{20}H_{22}N_4O_5$
Molecular weight: 398.42
CAS Registry No.: 59721-28-7
Merck Index: 1775



SAMPLE

Matrix: bile, duodenal fluid, pancreatic juice
Sample preparation: 500 µL Bile, pancreatic juice, or duodenal juice + 5 µL 100 µg/mL 4-guanidinobenzanilide methanesulfonate in water + 100 µL 2 M methanesulfonate, purify using a Sep-Pak C18 SPE cartridge.

HPLC VARIABLES

Column: 150 × 4.6 YMC-Pack A302 ODS (Shimakyu)
Mobile phase: MeOH:water:acetic acid:5% sodium dodecyl sulfate in 50% EtOH:5% sodium heptanoyl sulfate in 50% EtOH 200:140:1:1.5:4
Flow rate: 0.8
Detector: UV 265

OTHER SUBSTANCES

Extracted: metabolites

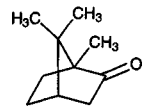
KEY WORDS

dog; SPE

REFERENCE

Kitagawa,M.; Hayakawa,T.; Kondo,T.; Shibata,T.; Sugimoto,Y. Pancreatic and biliary excretion of camostat in dogs, *Digestion*, **1988**, *39*, 204–209.

Camphor



Molecular formula: $C_{10}H_{16}O$
Molecular weight: 152.24
CAS Registry No.: 76-22-2
Merck Index: 1779

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 5 mL Plasma + 10 mL peroxide-free ether, mix. Remove the organic layer and add it to 2 mL isopropanol, evaporate the mixture to 2 mL under a stream of nitrogen at room temperature, add 2 mL of a saturated solution of 2,4-dinitrophenylhydrazine in MeOH, add 1 drop of concentrated HCl, heat at 85-90° for 18 h, cool, evaporate under a stream of nitrogen at 50° to 1 mL, add 2 mL 2 M HCl, evaporate to 2 mL, add 2 mL 2 M HCl, add 4 mL isooctane, rotate mechanically for 30 min. Remove the organic layer and wash it twice with 4 mL water, centrifuge, dry over anhydrous sodium sulfate, add to a florisil column, elute with 10 mL alcohol-free chloroform, evaporate the eluate to dryness under reduced pressure, reconstitute the residue in 2 mL MeCN:water 70:30, inject a 200 μL aliquot. Urine. Adjust 40 mL urine to pH 6.5 with three drops 6 M HCl, saturate with NaCl, extract with 10 mL peroxide-free ether. Remove the organic layer and add it to 2 mL isopropanol, evaporate the mixture to 2 mL under a stream of nitrogen at room temperature, add 2 mL of a saturated solution of 2,4-dinitrophenylhydrazine in MeOH, add 1 drop of concentrated HCl, heat at 85-90° for 18 h, cool, evaporate under a stream of nitrogen at 50° to 1 mL, add 2 mL 2 M HCl, evaporate to 2 mL, add 2 mL 2 M HCl, add 4 mL isooctane, rotate mechanically for 30 min. Remove the organic layer and wash it twice with 4 mL water, centrifuge, dry over anhydrous sodium sulfate, add to a florisil column, elute with 10 mL alcohol-free chloroform, evaporate the eluate to dryness under reduced pressure, reconstitute the residue in 2 mL MeCN:water 70:30, inject a 50 μL aliquot. (The florisil (60-100 mesh; J.T. Baker) was heated at 120° overnight, cooled, and 3% water added. The column was prepared by plugging the end of a Pasteur pipette with glass wool and adding florisil to a height of 6 cm followed by anhydrous sodium sulfate to a height of 1 cm, pre-wash with 3 mL isooctane.)

HPLC VARIABLES

Column: radial compression μBondapak C18
Mobile phase: MeCN:water 82:18
Flow rate: 1
Injection volume: 50-200
Detector: UV 368.5

CHROMATOGRAM

Retention time: 14.5
Limit of detection: 20 ng/mL (plasma), 10 ng/mL (urine)

KEY WORDS

plasma; horse; derivatization; SPE

REFERENCE

Gallicano,K.D.; Park,H.C.; Young,L.M. A sensitive liquid chromatographic procedure for the analysis of camphor in equine urine and plasma, *J.Anal.Toxicol.*, **1985**, *9*, 24–30.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 1 5 μm LiChrosorb RP18

Mobile phase: EtOH:water 35:65 containing 20 mM α-cyclodextrin and 0.5 mM tri-O-methyl-α-cyclodextrin
Column temperature: 25
Flow rate: 0.04
Injection volume: 20
Detector: UV 280

CHROMATOGRAM

Retention time: k' 7.9, k' 9.3 (enantiomers)

OTHER SUBSTANCES

Extracted: fenchone

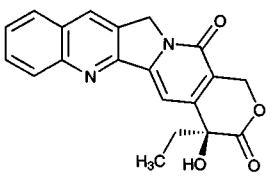
KEY WORDS

chiral

REFERENCE

Nowakowski,R.; Bielejewska,A.; Duszczyk,K.; Sybilska,D. Chiral discrimination by high-performance liquid chromatography with joint use of two cyclodextrin additives, *J.Chromatogr.A*, **1997**, *782*, 1–11.

Camptothecin



Molecular formula: $C_{20}H_{16}N_2O_4$
Molecular weight: 348.36
CAS Registry No.: 7689-03-4
Merck Index: 1783

SAMPLE

Matrix: blood
Sample preparation: Condition a C18 SPE cartridge (Waters) with 1 mL MeOH and 1 mL water. Vortex 100 μL plasma and 10 μL 20 nM IS in water for 5 s, add to the SPE cartridge, wash twice with 1 mL water, wash with 1 mL MeOH:water 20:80. Elute with 1 mL MeOH:25 mM pH 2.55 KH_2PO_4 75:25, store at -70°, thaw, inject an aliquot.

HPLC VARIABLES

Guard column: μBondapak
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:25 mM pH 4.8 KH_2PO_4 containing 1 mM sodium heptanesulfonate 35:65
Flow rate: 0.85
Detector: F ex 360 em 440

CHROMATOGRAM

Retention time: 6.0
Internal standard: 7-ethyl-10-hydroxycamptothecin (4.7)
Limit of quantitation: 5.74 nM

KEY WORDS

SPE; pharmacokinetics; plasma; procedure measures only lactone form of camptothecin

REFERENCE

Ahmed,F.; Vyas,V.; Saleem,A.; Li,X.-G.; Zamek,R.; Cornfield,A.; Haluska,P.; Ibrahim,N.; Rubin,E.H.; Gupta,E. High-performance liquid chromatographic quantitation of total and lactone 20(S)camptothecin in patients receiving oral 20(S)camptothecin, *J.Chromatogr.B*, **1998**, *707*, 227–233.

SAMPLE

Matrix: blood
Sample preparation: Vortex 100 μL plasma, 10 μL 20 nM IS in water, and 300 μL MeOH for 5 s, centrifuge at 5800 g for 3 min. Acidify the clear supernatant with 50 μL 500 mM hydrochloric acid, inject an aliquot.

HPLC VARIABLES

Guard column: μBondapak
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:25 mM pH 4.8 KH_2PO_4 containing 1 mM sodium heptanesulfonate 35:65
Flow rate: 0.85
Detector: F ex 360 em 440

CHROMATOGRAM

Retention time: 6.0
Internal standard: 7-ethyl-10-hydroxycamptothecin (4.7)
Limit of quantitation: 5.74 nM

KEY WORDS

pharmacokinetics; plasma; procedure measures total camptothecin (carboxylate and lactone)

REFERENCE

Ahmed,F.; Vyas,V.; Saleem,A.; Li,X.-G.; Zamek,R.; Cornfield,A.; Haluska,P.; Ibrahim,N.; Rubin,E.H.; Gupta,E. High-performance liquid chromatographic quantitation of total and lactone 20(S)camptothecin in patients receiving oral 20(S)camptothecin, *J.Chromatogr.B*, **1998**, *707*, 227–233.

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 100 μL MeOH:10 mM HCl 40:60 + 800 mg solid NaCl, extract with 7.5 mL MeCN:n-butyl chloride 20:80 for 5 min, centrifuge at 4000 g for 2 min. Rotate quickly by hand to break the gels, centrifuge at 4000 g for 5 min. Mix the organic layer with 50 μL DMSO, dry under a gentle stream of nitrogen at 60° to approximately 50 μL. Reconstitute the residue in 100 μL MeOH and 100 μL perchloric acid:water 1:500, vortex for 5 s, inject a 100 μL aliquot.

HPLC VARIABLES

Guard column: 4 × 4 5 μm LiChrospher 100 RP-18

Column: 100 × 4.6 5 μm Hypersil ODS

Mobile phase: MeOH:buffer 40:60 adjusted to pH 5.5 with HCl (Buffer was 100 mM ammonium acetate containing 10 mM tetrabutylammonium sulfate.)

Column temperature: 50

Flow rate: 1

Injection volume: 100

Detector: F ex 355 em 515

CHROMATOGRAM

Retention time: 6.5

Internal standard: camptothecin

OTHER SUBSTANCES

Extracted: irinotecan

Noninterfering: acetaminophen, alizapride, codeine, dexamethasone, domperidone, metoclopramide, morphine, ranitidine

KEY WORDS

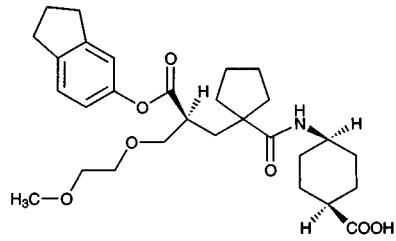
plasma; camptothecin is IS

REFERENCE

de Bruijn,P.; Verweij,J.; Loos,W.J.; Nooter,K.; Stoter,G.; Sparreboom,A. Determination of irinotecan (CPT-11) and its active metabolite SN-38 in human plasma by reversed-phase high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.B*, **1997**, *698*, 277–285.

Candoxatril

Molecular formula: $C_{29}H_{41}NO_7$
Molecular weight: 515.65
CAS Registry No.: 123122-55-4



SAMPLE
Matrix: blood
Sample preparation: Add 100 µL 2 M HCl to 1 mL plasma, extract with MTBE containing IS. Evaporate the organic phase, reconstitute the residue in 150 µL MeOH:2 mM ammonium acetate 50:50. Inject a 100 µL aliquot.

HPLC VARIABLES
Column: 30 × 4.6 C8
Mobile phase: MeOH:2 mM ammonium acetate 50:50
Flow rate: 1.0
Injection volume: 100
Detector: MS; PE SCIEX API III plus negative ion mode, m/z 398

CHROMATOGRAM
Retention time: 1.5
Internal standard: UK 77568 (Pfizer) (1.1, m/z 378)
Limit of quantitation: 2 ng/mL

OTHER SUBSTANCES
Extracted: candoxatrilat (active metabolite, 0.8, m/z 398)

KEY WORDS
plasma; mouse; rabbit; human

REFERENCE
Kaye,B.; Brearley,C.J.; Cussans,N.J.; Herron,M.; Humphrey,M.J.; Mollatt,A.R. Formation and pharmacokinetics of the active drug candoxatrilat in mouse, rat, rabbit, dog and man following administration of the prodrug candoxatril, *Xenobiotica*, **1997**, *27*, 1091–1102.

SAMPLE
Matrix: urine
Sample preparation: Inject a 500 µL aliquot directly.

HPLC VARIABLES
Column: 250 × 4.6 5 µm Inertsil ODS-2 (GL Sciences Inc.)
Mobile phase: Gradient. A was MeOH. B was 20 mM ammonium acetate. A:B from 0:100 to 100:0 over 20 min, maintain at 100:0 for 10 min.
Injection volume: 500
Detector: Radioactivity, Ramona-5 flow detector

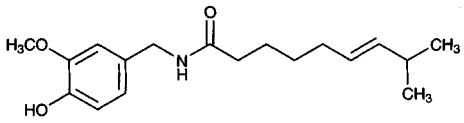
CHROMATOGRAM
Internal standard: UK 77568 (Pfizer)

OTHER SUBSTANCES
Extracted: candoxatrilat (active metabolite)

KEY WORDS
radiolabeled

REFERENCE
Kaye,B.; Brearley,C.J.; Cussans,N.J.; Herron,M.; Humphrey,M.J.; Mollatt,A.R. Formation and pharmacokinetics of the active drug candoxatrilat in mouse, rat, rabbit, dog and man following administration of the prodrug candoxatril, *Xenobiotica*, **1997**, *27*, 1091–1102.

Capsaicin



Molecular formula: $C_{18}H_{27}NO_3$
Molecular weight: 305.42
CAS Registry No.: 404-86-4
Merck Index: 1811

SAMPLE

Matrix: blood
Sample preparation: 200 μL Plasma + 300 μL acetone + 20 ng dihydrocapsaicin, homogenize, centrifuge at 13000 g for 2 min. Filter the supernatant (0.45 μm), inject a 5-20 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 Cosmosil 5Ph (Nakarai Chemicals)
Mobile phase: MeCN:100 mM KH_2PO_4 45:55, pH 5.0
Flow rate: 1
Injection volume: 5-20
Detector: E, Irica E-502, glassy carbon electrode + 750 mV, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 8.5
Internal standard: dihydrocapsaicin (10)
Limit of detection: 12 pg

KEY WORDS

plasma; rat; pharmacokinetics

REFERENCE

Kawada,T.; Watanabe,T.; Katsura,K.; Takami,H.; Iwai,K. Formation and metabolism of pungent principle of *Capsicum* fruits. XV. Microdetermination of capsaicin by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1985**, *329*, 99–105.

SAMPLE

Matrix: bulk
Sample preparation: Grind to pass 200 Mesh screen. Reflux 10 g in 100 mL acetone, cool, filter (Whatman No. 41 filter paper), evaporate the filtrate under reduced pressure. Dissolve the residue in 50 mL denatured EtOH, dilute a 5 mL aliquot to 10 mL with MeOH: water 50:50, filter (0.5 μm), inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm C8 (Supelco)
Mobile phase: Dissolve 1.85 g sodium pentanesulfonate in 400 mL water, make up to 1 L with MeOH.
Flow rate: 2
Injection volume: 20
Detector: UV 200

CHROMATOGRAM

Retention time: 6.5

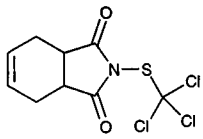
OTHER SUBSTANCES

Simultaneous: nordihydrocapsaicin, dihydrocapsaicin

REFERENCE

Weaver,K.M.; Awde,D.B. Rapid high-performance liquid chromatographic method for the determination of very low capsaicin levels, *J.Chromatogr.*, **1986**, *367*, 438–442.

Captan



Molecular formula: $C_9H_8Cl_3NO_2S$
Molecular weight: 300.59
CAS Registry No.: 133-06-2
Merck Index: 1815

SAMPLE

Matrix: formulations
Sample preparation: Add a 200 mg tablet containing 6 mg captan and 5 mg sulfur to 5 mL carbon disulfide, extract the solid residue five times with 5 mL carbon disulfide, combine the extracts and evaporate to constant weight. Dissolve the residue in 2 mL carbon disulfide, make up to 10 mL with MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4 10 μm Perkin-Elmer C8
Mobile phase: MeOH:water 90:10
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 3.2

OTHER SUBSTANCES

Simultaneous: sulfur

KEY WORDS

tablets

REFERENCE

Fedeli,G.; Moltrasio,D.; Aleotti,M.; Gazzani,G. High-performance liquid chromatographic determination of sulphur and captan in a mixture, *J.Chromatogr.*, **1988**, *447*, 263–267.

SAMPLE

Matrix: solutions
Sample preparation: Pass 100 mL water through column A at 5 mL/min then elute the contents of column A onto column B with the mobile phase, elute column B with the mobile phase and monitor the effluent fom column B.

HPLC VARIABLES

Column: A 30 × 4.6 5 μm Spherrisorb ODS C18; B 250 × 4.6 5 μm Supelcosil LC-8 C8
Mobile phase: Gradient. MeCN:water 30:70 for 5 min, to 60:40 over 10 min, maintain at 60:40 for 10 min, to 30:70 over 5 min, maintain at 30:70 for 5 min and inject next sample.
Flow rate: 1.5
Injection volume: 100000
Detector: UV 220

CHROMATOGRAM

Retention time: 21.10
Limit of detection: 460 pg/mL

OTHER SUBSTANCES

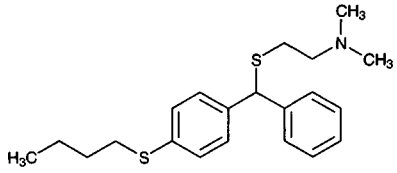
Simultaneous: propoxur, carbofuran, carbaryl, propham, chloropropham, barban, butylate

KEY WORDS
water; drinking water; column-switching

REFERENCE

Marvin,C.H.; Brindle,I.D.; Hall,C.D.; Chiba,M. Development of an automated high-performance liquid chromatographic method for the on-line pre-concentration and determination of trace concentrations of pesticides in drinking water, *J.Chromatogr.*, **1990**, *503*, 167–176.

Captodiamine



Molecular formula: $C_{21}H_{29}NS_2$
Molecular weight: 359.60
CAS Registry No.: 486-17-9, 904-04-1 (HCl)
Merck Index: 1816
Lednicer No.: 1 44

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 20.15

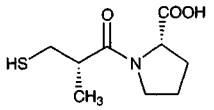
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Captopril



Molecular formula: $C_9H_{15}NO_3S$
Molecular weight: 217.29
CAS Registry No.: 62571-86-2
Merck Index: 1817
Lednicer No.: 3 128; 4 7, 58, 81, 128

SAMPLE

Matrix: blood
Sample preparation: Mix 100 μL plasma with 100 μL 100 mM pH 7.5 borate buffer and 10 μL 10% monobromobimane in MeCN, vortex for 15 s, let stand at room temperature for 5 min, add 200 μL MeCN, vortex, centrifuge at 3000 g for 5 min. Keep the supernatant at -20° prior to analysis. Inject a 10 μL aliquot.

HPLC VARIABLES

Guard column: μBondapak C 18 Guard-Pak
Column: 150 × 3.9 4 μm NovaPak C 18
Mobile phase: MeCN:water:trifluoroacetic acid 20:80:0.1
Column temperature: 50
Flow rate: 1.0
Injection volume: 10
Detector: F ex 400 em 480

CHROMATOGRAM

Retention time: 4.8
Limit of detection: 30 pg
Limit of quantitation: 12.5 ng/mL

KEY WORDS

plasma; derivatization; pharmacokinetics; human; rat

REFERENCE

Kok,R.J.; Visser,J.; Moolenaar,F.; De Zeeuw,D.; Meijer,D.K.F. Bioanalysis of captopril: two sensitive high-performance liquid chromatographic methods with pre-or postcolumn fluorescent labeling, *J.Chromatogr.B*, **1997**, *693*, 181–189.

SAMPLE

Matrix: blood
Sample preparation: Add 30 μL 1 mg/mL p-bromophenacyl bromide in MeOH and 20 μL 50 μg/mL IS in MeOH to 500 μL plasma. Mix for 30 s and let stand at room temperature for 20 min. Add 100 μL 1 M HCL, mix for 15-20 s. Extract with 4 mL ethyl acetate: benzene 50:50 (Caution! Benzene is a carcinogen!). Mix for 30 s and then shake gently for 5 min. Centrifuge and remove the organic layer, evaporate it to dryness, reconstitute the residue in 200 μL MeCN. Inject a 20 μL aliquot.

HPLC VARIABLES

Column: 30 × 0.4 10 μm μBondapak C18
Mobile phase: MeCN:water:acetic acid 52.5:47.1:0.4
Flow rate: 1
Injection volume: 20
Detector: UV 260

CHROMATOGRAM

Retention time: 4.39

Internal standard: captopril-DDPM adduct (Prepare captopril-DDPM adduct as follows. Weigh 150 mg captopril and 250 mg N-(4-dimethylamino-3,5-dinitrophenyl)maleimide (DDPM), add 40 ml 20 mM pH 7.0 phosphate buffer and 30 mL acetone. Heat in a water-bath for 15 min and let stand at room temperature overnight. Evaporate to dryness under vacuum and add 100 mL water. Make alkaline with 2 M NaOH (the color changes from orange to deep red) and wash 4 times with 25 mL portions of ethyl acetate. Acidify the aqueous layer with 1 M HCl and extract twice with 25 mL portions of ethyl acetate. Dry the organic layer over magnesium sulfate and evaporate to yield 300 mg captopril-DDPM adduct as a deep red oil. Dissolve in 10-15 mL ethyl acetate and precipitate with 100 mL hexane.) (3.62)
Limit of detection: 5 ng/mL

KEY WORDS
plasma; derivatization

REFERENCE
Klein,J.; Colin,P.; Scherer,E.; Levy,M.; Koren,G. Simple measurement of captopril in plasma by high-performance liquid chromatography with ultraviolet detection, *Ther.Drug Monit.*, **1990**, *12*, 105–110.

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg Bakerbond C18 SPE cartridge with two 200 μL portions of MeOH and two 200 μL portions of water. 1 mL Plasma + 100 μL 200 mM disodium EDTA + 100 μL 200 mM ascorbic acid + 200 μL 1 μg/mL IS + 400 μL 3 M perchloric acid, mix, centrifuge at 4000 g for 15 min, wash the precipitate three times with 500 μL portions of water. Combine the supernatant and the washings and neutralize (indicator paper) them with 1 M NaOH, add 3 mL 1 M pH 8.2 Tris buffer, add 100 μL 20 μg/mL 1-benzyl-2-chloropyridinium bromide, let stand for 15 min, adjust pH to 2.5-3.0 (indicator paper) with 4 M phosphoric acid, centrifuge, add to the SPE cartridge, wash with 1 mL water, dry under vacuum suction for 10 min, wash with three 100 μL portions of MeCN, dry under vacuum for 5 min, elute with 200 μL MeOH:acetic acid 80:20, elute with two 200 μL portions of MeOH:water 80:20. Combine the eluates and evaporate them to dryness at 60°, reconstitute with 50 μL mobile phase, inject a 20 μL aliquot. To measure total captopril proceed as follows. 1 mL Plasma + 100 μL 200 mM disodium EDTA + 100 μL 200 mM ascorbic acid + 200 μL 1 μg/mL IS + 2 mL 100 mM perchloric acid + 100 μL 40 mg/mL triphenylphosphine in MeCN, mix, heat at 50° for 40 min, cool, add 400 μL 3 M perchloric acid, mix, centrifuge at 4000 g for 15 min, wash the precipitate three times with 500 μL portions of water. Combine the supernatant and the washings and neutralize (indicator paper) them with 1 M NaOH, add 3 mL 1 M pH 8.2 Tris buffer, add 100 μL 20 μg/mL 1-benzyl-2-chloropyridinium bromide, let stand for 15 min, adjust pH to 2.5-3.0 (indicator paper) with 4 M phosphoric acid, centrifuge, add to the SPE cartridge, wash with 1 mL water, dry under vacuum suction for 10 min, wash with three 100 μL portions of MeCN, dry under vacuum for 5 min, elute with 200 μL MeOH:acetic acid 80:20, elute with two 200 μL portions of MeOH:water 80:20. Combine the eluates and evaporate them to dryness at 60°, reconstitute with 50 μL mobile phase, inject a 20 μL aliquot. To measure protein-conjugated captopril proceed as follows. 1 mL Plasma + 100 μL 200 mM disodium EDTA + 100 μL 200 mM ascorbic acid + 200 μL 1 μg/mL IS + 400 μL 3 M perchloric acid, mix, centrifuge at 4000 g for 15 min, wash the precipitate three times with 500 μL portions of water. Suspend the precipitate in 2 mL 100 mM perchloric acid, add 200 μL 1 μg/mL IS, add 100 μL 40 mg/mL triphenylphosphine in MeCN, heat at 50° with occasional shaking for 40 min, cool, add 400 μL 3 M perchloric acid, mix, centrifuge at 4000 g for 15 min, wash the precipitate three times with 500 μL portions of water. Combine the supernatant and the washings and neutralize (indicator paper) them with 1 M NaOH, add 3 mL 1 M pH 8.2 Tris buffer, add 100 μL 20 μg/mL 1-benzyl-2-chloropyridinium bromide, let stand for 15 min, adjust pH to 2.5-3.0 (indicator paper) with 4 M phosphoric acid, centrifuge, add to the SPE cartridge, wash with 1 mL water, dry under vacuum suction for 10 min, wash with three 100 μL portions of MeCN, dry under vacuum for 5 min, elute with 200 μL MeOH:acetic acid 80:20, elute with two 200 μL portions of MeOH:

water 80:20. Combine the eluates and evaporate them to dryness at 60°, reconstitute with 50 μL mobile phase, inject a 20 μL aliquot. (Prepare 1-benzyl-2-chloropyridinium bromide as follows. Add 8.5 g benzyl bromide to 4.5 g 2-chloropyridine with stirring, stir at 60° overnight, cool, filter, wash with acetone, dry under vacuum to give 1-benzyl-2-chloropyridinium bromide (mp 187-191°).)

HPLC VARIABLES
Guard column: 20 × 2.1 5 μm Hypersil
Column: 100 × 2.1 5 μm ODS Hypersil
Mobile phase: Acetone:buffer 25:75 (Buffer was 200 mM pH 2.5 citrate buffer containing 10 mM sodium octanesulfonate and 15 mM NaCl.)
Column temperature: 50
Flow rate: 0.2
Injection volume: 20
Detector: UV 314

CHROMATOGRAM
Retention time: 10
Internal standard: 1-benzyl-2-chloro-4-methylpyridinium bromide-captopril adduct (Preparation is as follows. Add 8.5 g benzyl bromide to 5.1 g 2-chloro-4-methylpyridine (Loba-Chemie, Vienna) with stirring, stir at 60° overnight, cool, filter, wash with acetone, dry under vacuum to give 1-benzyl-2-chloro-4-methylpyridinium bromide. Condition a 1 g C18 SPE cartridge with two 1 mL portions of MeOH and with 1 mL water. Stir 30 mg captopril and 80 mg 1-benzyl-2-chloro-4-methylpyridinium bromide in 5 mL 500 mM pH 8.2 Tris buffer for 1 h, add 1.6 mL 100 mM sodium sulfide, let stand for 10 min, add to the SPE cartridge, wash with two 1 mL portions of water, dry under vacuum for 10 min, wash with three 1 mL portions of MeCN, dry under vacuum for 5 min, elute with 5 mL MeOH:water 80:20. Dilute the eluate to 10 mL with water, dilute with water to an adduct concentration of 3 μg/mL. 2-Chloro-4-methylpyridine can also be prepared as follows. Stir 3.6 g 2-amino-4-picoline (2-amino-4-methylpyridine) in 75 g concentrated HCl at between -15° and -20°, saturate with hydrogen chloride gas, add 3.5 g pulverized sodium nitrite in small portions, let stand overnight, neutralize, steam distil to give 2-chloro-4-methylpyridine as a colorless liquid (bp 194-195°) (Ber. 1924, 57, 791).) (12)
Limit of quantitation: 10 ng/mL

KEY WORDS
derivatization; plasma; SPE

REFERENCE
Bald,E.; Sypniewski,S.; Drzewoski,J.; Stepien,M. Application of 2-halopyridinium salts as ultraviolet derivatization reagents and solid-phase extraction for determination of captopril in human plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *681*, 283–289.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 150 μL 1 mg/mL p-bromophenacyl bromide in MeOH + 20 μL 50 μg/mL 4-chloro-2-nitroaniline in MeOH, vortex for 30 s, let stand at room temperature for 15 min, add 200 μL 2 M HCl, vortex for 10 s, add 5 mL benzene:ethyl acetate 50:50 (Caution! Benzene is a carcinogen!), vortex for 2 min. Remove a 4.5 mL aliquot of the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 100 μL MeCN, vortex for 20 s, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Spherisorb C18
Mobile phase: MeCN:water:acetic acid 44:55:0.2
Flow rate: 1.4
Injection volume: 25
Detector: UV 258

CHROMATOGRAM
Retention time: 3.6
Internal standard: 4-chloro-2-nitroaniline (4.7)
Limit of detection: 2 ng/mL
Limit of quantitation: 5 ng/mL

KEY WORDS
derivatization; plasma; pharmacokinetics

REFERENCE
Li,K.; Tan,L.; Zhou,J.A. HPLC determination of captopril in human plasma and its pharmacokinetic study, *Biomed.Chromatogr.*, **1996**, *10*, 237–239.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL silica SPE cartridge with 1 mL benzene (Caution! Benzene is a carcinogen!). Collect 10 mL blood in a tube containing 30 mg 2,4'-dibromoacetophenone (p-bromophenacyl bromide), vortex for 30 s, let stand at room temperature for at least 15 min. Remove the serum and freeze it at -20°. Thaw serum and add 1 mL to 200 μL 1 M HCl, mix, extract twice with 2 mL portions of benzene. Add the organic layers to the SPE cartridge, wash with 4 mL benzene, elute with 500 μL MeCN, add 50 μL 10 μg/mL IS in acetone to the eluate, make up to 1 mL with mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: Spheri-5 ODS-GU
Column: 100 × 4.6 5 μm Spheri-5 ODS
Mobile phase: MeCN:water:acetic acid 45:54:1
Flow rate: 1
Injection volume: 100
Detector: UV 263

CHROMATOGRAM
Retention time: 4
Internal standard: thiosalicylic acid-p-bromophenacyl bromide adduct (Prepare by dissolving 2.4 mmoles thiosalicylic acid and 2.4 mmoles p-bromophenacyl bromide in 40 mL MeOH, adjust to pH 7 by the dropwise addition of 1 M NaOH, allow to stand at room temperature for 10 min, evaporate to dryness under reduced pressure, reconstitute with 40 mL 50 mM pH 7.0 phosphate buffer, wash twice with 20 mL portions of hexane, adjust pH to 2 with dilute HCl, extract with 40 mL ethyl acetate, evaporate to dryness under reduced pressure, recrystallize the residue from benzene to give the adduct as pale yellow plates (Chem. Pharm. Bull. 1981, 29, 150).) (8)
Limit of detection: 15 ng/mL

KEY WORDS
derivatization; SPE; whole blood; pharmacokinetics

REFERENCE
Bahmaei,M.; Khosravi,A.; Zamiri,C.; Massoumi,A.; Mahmoudian,M. Determination of captopril in human serum by high performance liquid chromatography using solid-phase extraction, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 1181–1186.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. Free captopril determination. Mix 100 μL plasma with 100 μL 100 mM pH 7.5 borate buffer and 300 μL MeOH, vortex, centrifuge at 3000 g for 5 min, inject a 25 μL aliquot directly. Plasma, urine. Total captopril determination. Mix

100 μL plasma or diluted urine (1:10) with 100 μL 100 mM pH 7.5 borate buffer and 100 μL 1% tributylphosphine in MeOH, vortex for 15 s, allow to stand at room temperature for 15 min, add 200 μL MeOH, vortex, centrifuge at 3000 g for 5 min. Let the supernatant stand at room temperature for at least 1 h. Inject a 25 μL aliquot.

HPLC VARIABLES
Guard column: μBondapak C 18 Guard-Pak
Column: 150 × 3.9 4 μm NovaPak C 18
Mobile phase: MeCN:water:trifluoroacetic acid 15:85:0.1 containing 100 mg/L glycine
Column temperature: 50
Flow rate: 0.8
Injection volume: 25
Detector: F ex 345 em 455 following post-column reaction. The column effluent mixed with 100 mM pH 8.5 borate buffer containing 100 mg/L o-phthalaldehyde pumped at 1.2 mL/min and this mixture flowed through a 1 m × 0.5 mm I.D. mixing coil to the detector.

CHROMATOGRAM
Retention time: 5.4
Limit of detection: 50 pg (plasma)
Limit of quantitation: 25 ng/mL (plasma), 250 ng/mL (urine)

KEY WORDS
plasma; post-column reaction; pharmacokinetics; human; rat

REFERENCE
Kok,R.J.; Visser,J.; Moolenaar,F.; De Zeeuw,D.; Meijer,D.K.F. Bioanalysis of captopril: two sensitive high-performance liquid chromatographic methods with pre-or postcolumn fluorescent labeling, *J.Chromatogr.B*, **1997**, *693*, 181–189.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a 100 mg Bakerbond C18 SPE cartridge with two 200 μL portions of MeOH and two 200 μL portions of water. Whole blood. 3 mL Whole blood + 100 μL 100 mM EDTA + 100 μL 200 mM ascorbic acid + 2 mL 1 M pH 8.2 Tris buffer + 200 μL 3 μg/mL IS + 100 μL 20 μg/mL 1-benzyl-2-chloropyridinium bromide, vortex for 15 min, centrifuge at 3000 g for 10 min. Remove a 1 mL aliquot of the supernatant and add it to 400 μL 3 M perchloric acid, centrifuge for 15 min, rinse the precipitate with 500 μL portions of water. Combine the supernatant and the rinses and adjust the pH to 2.5-3.0 (indicator paper) with 100 mM NaOH, add to the SPE cartridge, wash with 1 mL water, dry under vacuum suction for 10 min, elute with 200 μL MeOH:acetic acid 80:20, elute with two 200 μL portions of MeOH:water 80:20. Combine the eluates and evaporate them to dryness at 60°, reconstitute with 50 μL water, inject a 20 μL aliquot. Urine. 500 μL Urine + 100 μL 200 mM EDTA + 100 μL 200 mM ascorbic acid + 3 mL 1 M pH 8.2 Tris buffer + 200 μL 3 μg/mL IS + 100 μL 20 μg/mL 1-benzyl-2-chloropyridinium bromide, vortex for 15 min, adjust the pH to 2.5-3.0 with 4 M phosphoric acid, add to the SPE cartridge, wash with 1 mL water, dry under vacuum suction for 10 min, elute with 200 μL MeOH:acetic acid 80:20, elute with two 200 μL portions of MeOH:water 80:20. Combine the eluates and evaporate them to dryness at 60°, reconstitute with 50 μL water, inject a 20 μL aliquot. (Prepare 1-benzyl-2-chloropyridinium bromide as follows. Add 8.5 g benzyl bromide to 4.5 g 2-chloropyridine with stirring, stir at 60° overnight, cool, filter, wash with acetone, dry under vacuum to give 1-benzyl-2-chloropyridinium bromide (mp 187-191°) (J. Chromatogr. B 1996, 681, 283).)

HPLC VARIABLES
Guard column: 20 × 2.1 5 μm Hypersil
Column: 150 × 3.3 7 μm Separon SGX (Struzeni, Prague)
Mobile phase: Gradient. A was MeCN:100 mM pH 2.5 citric acid buffer containing 20 mM sodium octanesulfonate 25:75. B was MeCN:MeOH 50:50. A:B 100:0 for 10 min, to 80:20

over 10 min, maintain at 80:20 for 5 min, to 60:40 over 5 min, return to initial conditions over 7 min.
Column temperature: 50
Flow rate: 0.5
Injection volume: 20
Detector: UV 314

CHROMATOGRAM

Retention time: 23
Internal standard: 1-benzyl-2-chloro-4-methylpyridinium bromide-captopril adduct (Preparation is as follows. Add 8.5 g benzyl bromide to 5.1 g 2-chloro-4-methylpyridine (Loba-Chemie, Vienna) with stirring, stir at 60° overnight, cool, filter, wash with acetone, dry under vacuum to give 1-benzyl-2-chloro-4-methylpyridinium bromide. Condition a 1 g C18 SPE cartridge with two 1 mL portions of MeOH and with 1 mL water. Stir 30 mg captopril and 80 mg 1-benzyl-2-chloro-4-methylpyridinium bromide in 5 mL 500 mM pH 8.2 Tris buffer for 1 h, add 1.6 mL 100 mM sodium sulfide, let stand for 10 min, add to the SPE cartridge, wash with two 1 mL portions of water, dry under vacuum for 10 min, wash with three 1 mL portions of MeCN, dry under vacuum for 5 min, elute with 5 mL MeOH: water 80:20. Dilute the eluate to 10 mL with water, dilute with water to an adduct concentration of 3 μg/mL (J. Chromatogr. B 1996, 681, 283). 2-Chloro-4-methylpyridine can also be prepared as follows. Stir 3.6 g 2-amino-4-picoline (2-amino-4-methylpyridine) in 75 g concentrated HCl at between -15° and -20°, saturate with hydrogen chloride gas, add 3.5 g pulverized sodium nitrite in small portions, let stand overnight, neutralize, steam distil to give 2-chloro-4-methylpyridine as a colorless liquid (bp 194-195°) (Ber. 1924, 57, 791).) (27)
Limit of detection: 0.3 ng/mL
Limit of quantitation: 10 ng/mL

KEY WORDS

derivatization; whole blood; SPE

REFERENCE

Sypniewski,S.; Bald,E. Determination of captopril and its disulphides in whole human blood and urine by high-performance liquid chromatography with ultraviolet detection and precolumn derivatization, *J.Chromatogr.A*, **1996**, *729*, 335–340.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A: B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30

Detector: UV 200.5

CHROMATOGRAM
Retention time: 9.652

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Dissolve solutions, powders, or powdered tablets in water to give a 200-325 μM solution, filter if necessary. Mix 500 μL solution with 500 μL 170 mM pH 7.5 borate buffer and 500 μL 2 mM 1,1-bis(phenylsulfonyl)ethylene (1,1'-ethenylidenebis(sulfonyl)bis-benzene; Fluka, Merck) in MeOH, let stand at room temperature for 2 min, add 500 μL water, add 300 μL chloroform, vortex for 1 min, centrifuge for 2 min. Remove a 1 mL aliquot of the aqueous layer and add it to 500 μL 300 mM orthophosphoric acid, add 100-200 μL 30 μM IS in MeCN, mix, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Hypersil C18
Mobile phase: MeCN:buffer 51:49 (Buffer was 50 mM pH 4.0 triethylamine-phosphate buffer.)
Flow rate: 1
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 3,5
Internal standard: 1(2H)-acenaphthylenone (Synthesis is as follows. Prepare dichloroacenaphthylenone by refluxing acenaphthenequinone with phosphorus pentachloride in anhydrous toluene. Mix 200 g glacial acetic acid, 20 g crude dichloroacenaphthylenone, and 30 g iron powder, heat gently until refluxing, heat for about 3 h, dilute with 500 mL water, steam distil, filter the distillate to recover the product, recrystallize from benzene (Caution! Benzene is a carcinogen!) to obtain 1(2H)-acenaphthylenone (mp 119-121°) (Gazz. Chim. Italia 1938, 68, 184).) (5.5)
Limit of detection: 100 pmole

KEY WORDS
derivatization; tablets; powders

REFERENCE
Cavrini,V.; Gotti,R.; Andrisano,V.; Gatti,R. 1,1'-[Ethylidenebis(sulfonyl)]bis-benzene: A useful pre-chromatographic derivatization reagent for HPLC analyses of thiol drugs, *Chromatographia*, **1996**, *42*, 515–520.

SAMPLE
Matrix: intestinal mucosal homogenate
Sample preparation: 400 μL Homogenate mixture + 400 μL 1 M HCl, mix, centrifuge at 4° at 34000 g for 10 min, filter (0.45 μm) the supernatant, inject an aliquot of the filtrate.

HPLC VARIABLES
Guard column: 20 mm long Supelguard LC-18S (Supelco)

Column: 250 × 4.6 Suplecosil LC-18S
Mobile phase: MeOH:water:85% phosphoric acid 54.97:44.98:0.05
Flow rate: 1
Detector: UV 210

KEY WORDS
rat

REFERENCE
Sinko,P.J.; Hu,P. Determining intestinal metabolism and permeability for several compounds in rats. Implications on regional bioavailability in humans, *Pharm.Res.*, **1996**, *13*, 108–113.

SAMPLE
Matrix: solutions
Sample preparation: Add 90 μL cold 10% trichloroacetic acid (containing 1 mM disodium EDTA) to 10 μL blood. Centrifuge at 1850 g at 0° for 5 min. Dilute a 10 μL aliquot of the supernatant with 2 mL water. Mix a 200 μL aliquot of the supernatant with 100 μL 100 μM o-phthalaldehyde reagent and 100 μL 200 μM N-(4-aminobutyl)-N-ethylisoluminol reagent, vortex thoroughly. Let stand for about 2 min. Inject a 20 μL aliquot of the reaction mixture. (Prepare reagents as follows. Dissolve o-phthalaldehyde in 50 mM pH 9.0 sodium borate buffer containing 100 mM potassium dihydrogen phosphate to give a 100 μM solution. Prepare a 1 mM solution of N-(4-aminobutyl)-N-ethylisoluminol (chemiluminescence-grade, Tokyo Kasei) in MeOH containing 5 mM HCl. Dilute with MeOH to give a 200 μM solution.)

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrosorb RP-18
Column: 150 × 4.6 5 μm Cosmosil 5C18-AR (Nacalai Tesque)
Mobile phase: MeOH:THF:100 mM pH 7.5 phosphate buffer 25:5:70
Flow rate: 1
Injection volume: 20
Detector: Chemiluminescence, TOA Electronics ICA-3070 detector following post-column reaction. The column effluent mixed with 150 mM hydrogen peroxide pumped at 0.2 mL/min and 25 μM hematin in 150 mM sodium carbonate buffer pumped at 3 mL/min and this mixture flowed through a 200 × 0.5 mm PTFE reaction coil to the detector.

CHROMATOGRAM
Retention time: k' 13.3

OTHER SUBSTANCES
Extracted: acetylcysteine

KEY WORDS
post-column reaction; derivatization

REFERENCE
Sano,A.; Nakamura,H. Chemiluminescence detection of thiols by high-performance liquid chromatography using o-phthalaldehyde and N-(4-aminobutyl)-N-ethylisoluminol as precolumn derivatization reagents, *Anal.Sci.*, **1998**, *14*, 731–735.

SAMPLE
Matrix: solutions
Sample preparation: Mix a 200 μM solution in buffer with three volumes of a 400 μM solution of 5,5'-dithio-(bis-2-nitrobenzoic acid) in buffer, let stand at room temperature for 30 min, inject a 75 μL aliquot. (Buffer was 125 mM NaH_2PO_4 containing 154 mM NaCl, pH adjusted to 7.4 with NaOH.)

HPLC VARIABLES
Column: 250 × 4.6 Hypersil ODS1
Mobile phase: Gradient. MeCN:buffer 0:100 for 20 min, to 17.5:82.5 over 40 min. (Buffer was 125 mM NaH_2PO_4 containing 154 mM NaCl, pH adjusted to 7.4 with NaOH.)
Flow rate: 0.25 for 20 min, to 1 over 40 min
Injection volume: 75
Detector: UV 357

CHROMATOGRAM
Retention time: 61

OTHER SUBSTANCES
Simultaneous: N-acetylcysteine, N-acetylpenicillamine, cysteine, glutathione, penicillamine, thiomalic acid

KEY WORDS
derivatization

REFERENCE
Russell,J.; McKeown,J.A.; Hensman,C.; Smith,W.E.; Reglinski,J. HPLC determination of biologically active thiols using pre-column derivatization with 5,5'-dithio-(bis-2-nitrobenzoic acid), *J.Pharm.Biomed.Anal.*, **1997**, *15*, 1757–1763.

SAMPLE
Matrix: urine
Sample preparation: Dilute 10 mL urine to 15 mL with water, add to Extrelut-20 SPE cartridge, elute with 60 mL ethyl acetate:isopropanol 85:15. Evaporate under vacuum at 50°, filter, dry under nitrogen, reconstitute the residue in 100 μL acetone. Add 100 μL 1 mg/mL 3-bromomethylpropylphenazone in acetone, mix with 1 mg anhydrous potassium carbonate, make up to 200 μL with acetone. Let stand at 105 ± 5° for 30 min (for captopril) or 60 min (for hydrochlorothiazide or for the mixture). Cool the reaction mixture, dry under a gentle stream of nitrogen. Reconstitute the residue with 500 μL MeCN, shake for 2 min. inject a 10 μL aliquot. (3-Bromomethylpropylphenazone is produced by the reaction of propylphenazone with bromine and recrystallized from chloroform:diethyl ether 1:2. (Caution! Chloroform is a carcinogen!))

HPLC VARIABLES
Column: 250 × 4.6 6 μm Zorbax C8
Mobile phase: MeCN:MeOH:50 mM sodium acetate 34:8:28, adjusted to pH 6.5 with acetic acid
Column temperature: 35
Flow rate: 1
Injection volume: 10
Detector: UV 254

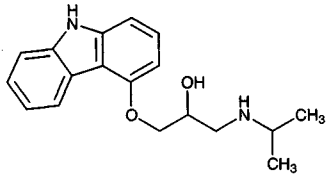
CHROMATOGRAM
Retention time: 8.6

OTHER SUBSTANCES
Extracted: hydrochlorothiazide

KEY WORDS
derivatization; SPE

REFERENCE
Khedr,A.; El-Sherief,H. 3-Bromomethyl-propyphenazone as a new derivatization reagent for high performance liquid chromatography of captopril and hydrochlorothiazide with UV-detection, *Biomed.Chromatogr.*, **1998**, *12*, 57–60.

Carazolol



Molecular formula: $C_{18}H_{22}N_2O_2$
Molecular weight: 298.38
CAS Registry No.: 57775-29-8
Merck Index: 1822

SAMPLE

Matrix: tissue

Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 5 mL MeOH and 5 mL water. Homogenize kidney with a kitchen grinder. Weigh out a 5 g sample and add 20 mL MeCN with continuous gentle mixing, mix vigorously on a vibromixer at 1500 rpm for 30 s, sonicate for 2 min, centrifuge at 4000 g for 5 min. Mix 7.5 mL sample extract and 40 mL 10% NaCl and add to SPE cartridge, wash with 1 mL 10 mM sulfuric acid, wash with 2 mL air, elute with 2 mL acidic MeCN. Place eluate in a washed tube and evaporate to 300 µL at 70° under a stream of nitrogen, mix gently, add 1 mL n-hexane, mix on a vibromixer for 30 s, centrifuge at 2000 g, inject a 50 µL aliquot of the aqueous phase. (Acidic MeCN was 1 mL 50 mM sulfuric acid and 100 mL MeCN. The washed tube was prepared by rinsing with concentrated ammonia, water, and acetone and drying under a stream of nitrogen.)

HPLC VARIABLES

Guard column: 10 × 2.1 37-50 µm Bondapak C18
Column: 300 × 3.9 Bondapak C18
Mobile phase: MeCN:water 55:45 containing 2.46 g/L anhydrous sodium acetate, pH adjusted to 6.5 with acetic acid
Flow rate: 1.2
Injection volume: 50
Detector: F ex 246 em 351

CHROMATOGRAM

Retention time: 5
Limit of detection: 0.3 ng/g

OTHER SUBSTANCES

Extracted: azaperol, chlorpromazine, acepromazine, xylazine, azaperone, haloperidol, propiomazine

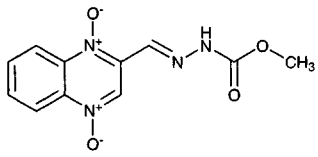
KEY WORDS

SPE; pig; kidney

REFERENCE

Keukens,H.J.; Aerts,M.M.L. Determination of residues of carazolol and a number of tranquilizers in swine kidney by high-performance liquid chromatography with ultraviolet and fluorescence detection, *J.Chromatogr.*, **1989**, *464*, 149–161.

Carbadox



Molecular formula: $C_{11}H_{10}N_4O_4$
Molecular weight: 262.22
CAS Registry No.: 6804-07-5
Merck Index: 1825

SAMPLE

Matrix: blood, eggs, tissue

Sample preparation: Blend 10 g muscle, liver, kidney, plasma, or eggs with 40 mL MeCN:MeOH for 3 min (stomacher blender), centrifuge at 2000 g for 5 min, pass the supernatant through a 400 × 10 glass column containing 8 g alumina (Woelm neutral, activity 1, lower layer) and 2 g florisil (75-150 μm, upper layer), collect the eluate. Evaporate 10 mL of the eluate to 0.9-1.1 mL under a stream of nitrogen at 40-50°, dilute to 4 mL with water, mix, extract with 2 mL isooctane, centrifuge at 2000 g for 5 min, inject 1 mL of the aqueous phase onto column A with mobile phase A, after 20 min backflush the contents of column A onto column B with mobile phase B, after 5 min remove column A from the circuit, monitor the effluent from column B.

HPLC VARIABLES

Column: A 10 × 2.1 column was slurry-packed with 55-105 μm material from a C18 Sep-Pak, 20 μm screens were used; B 10 × 2.1 37-50 μm Bondapak C18/Corasil + 100 × 3 5 μm ChromSpher C18 (Chrompack)

Mobile phase: A water; B MeCN:10 mM pH 6 acetate buffer 14:86

Flow rate: A 0.3; B 0.5

Injection volume: 1000

Detector: UV 390 following post-column derivatization. The column effluent was mixed with 0.5 M NaOH pumped at 0.23 mL/min. The mixture flowed through a 2 m × 0.5 mm i.d. knitted PTFE reaction coil to the detector.

CHROMATOGRAM

Retention time: 9

Limit of detection: 1 ng/g

OTHER SUBSTANCES

Extracted: metabolites

Noninterfering: chloramphenicol, chlortetracycline, clopidol, dapsone, decoquinate, dimetridazole, dinitolmide, doxycycline, ethopabate, fenbendazole, furaltadone, furazolidone, furnicozone, halofuginone, ipronidazole, methylbenzoquate, nicarbazin, nifursol, nitrofurantoin, nitrofurazone, nitrovin, olaquindox, oxytetracycline, pyrantel, robenidine, ronidazole, sulfadiazine, sulfanilamide, sulfadimethoxine, sulfadoxine, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfaquinoxaline, tetracycline, thiophanate, trimethoprim

KEY WORDS

plasma; column-switching; post-column reaction; derivatization; use yellow light and amber glassware; muscle; liver; kidney; pig

REFERENCE

Binnendijk,G.M.; Aerts,M.M.L.; Keukens,H.J.; Brinkman,U.A.T. Optimization and ruggedness testing of the determination of residues of carbadox and metabolites in products of animal origin. Stability studies in animal tissues, *J.Chromatogr.*, **1991**, *541*, 401–410.

SAMPLE

Matrix: feed

Sample preparation: Grind feed to pass 20 mesh. 10 g Feed + 5 mL water, swirl, let stand for 5 min, add 50 mL DMF:water 95:5, shake vigorously for 15 s, let stand in the dark

at room temperature overnight, filter (paper). Add 15 mL of the filtrate to 5 g alumina (Alcoa F-20, 80-200 mesh) in a 300 × 10 glass column, discard first several mL of eluate, collect remaining eluate, inject an aliquot

HPLC VARIABLES
Guard column: 100 × 2 μBondapak C18/Corasil
Column: 300 × 4 μBondapak C18
Mobile phase: MeCN:1% acetic acid 20:80
Detector: UV 280, UV 365

CHROMATOGRAM
Retention time: 4

OTHER SUBSTANCES
Extracted: furazolidone, nitrofurazone

KEY WORDS
protect from light

REFERENCE
Thorpe,V.A. Sample preparation of carbadox, furazolidone, nitrofurazone, and ethopabate in medicated feeds for high pressure liquid chromatography, *J.Assoc.Off.Anal.Chem.*, **1980**, *63*, 981–984.

SAMPLE
Matrix: feed
Sample preparation: Grind feed to pass 20 mesh sieve. 10 g Ground feed + 15 mL water, mix well, let stand for 5 min, add 25 mL MeCN:MeOH 50:50, shake vigorously for 30 min, centrifuge or filter (Whatman glass fiber GFA), pass 15 mL supernatant or filtrate through 4 g alumina in a 10 mm dia column, collect the first 4 mL eluate, inject a 25 μL aliquot. (Prepare alumina as follows. Stir 200 g Fisher neutral alumina (A-950) in 1 L water for 30 min, pour off fines, resuspend, filter (Whatman glass fiber GFA), dry with vacuum, wash 3 times with MeOH, dry at 80° overnight, store in desiccator.)

HPLC VARIABLES
Guard column: 30-40 μm pellicular C18 (Waters)
Column: 100 × 5 C18 radial compression (Waters)
Mobile phase: MeCN:buffer 18:82 (Buffer was 25 mL dibutylamine acetate made up to 1 L with water, pH 3.7. Dibutylamine acetate was prepared by titrating 100 mL dibutylamine to pH 2.5 with acetic acid (ca. 270 mL).)
Flow rate: 2
Injection volume: 25
Detector: UV 365

CHROMATOGRAM
Retention time: 2.4

OTHER SUBSTANCES
Simultaneous: pyrantel (UV 313)

KEY WORDS
protect from light

REFERENCE
Lowie,D.M.,Jr.; Teague,R.T.,Jr.; Quick,F.E.; Foster,C.L. High pressure liquid chromatographic determination of carbadox and pyrantel tartrate in swine feed and supplements, *J.Assoc.Off.Anal.Chem.*, **1983**, *66*, 602–605.

SAMPLE

Matrix: feed

Sample preparation: Grind feed to pass 2 mm sieve. 50 g Ground feed + 200 mL extraction solution, shake mechanically for 30 min, centrifuge at 1500 rpm for 5 min. Add 100 mL of the supernatant to a Celite column, rinse apparatus with 70 mL extraction solution, add rinses to Celite column. Collect all the eluates and add them to alumina column A, rinse apparatus with 20-30 mL extraction solution, add rinses to alumina column A. Collect all the eluate and evaporate just to dryness under reduced pressure, take up the residue in 50 mL chloroform. Extract with 10 mL 1 M NaOH (30 s shake), add 12 mL 1 M HCl to the aqueous phase, extract three times with 50 mL chloroform. Combine the extracts and dry them over 35-40 g anhydrous sodium sulfate. Evaporate to dryness and take up the residue in 5 mL DMF, add to alumina column B, rinse apparatus with 10 mL DMF, add rinses to column, wash with three 10 mL portions of DMF, wash with three 25 mL portions of chloroform, elute with chloroform:MeOH 75:25. Evaporate the eluate just to dryness, take up the residue in 2 mL MeOH:water 30:70, inject a 20 μL aliquot. (Extraction solution was chloroform:acidified MeOH 75:25. Acidified MeOH was MeOH containing 1% HCl. The Celite column was a 300 × 22 glass column containing 8-10 g acid-washed Celite 545, prewet with 50 mL extraction solution. Alumina column A was a 400 × 22 glass column containing 15 cm of alumina (Fisher A-540, 80-200 mesh), prewet with 50 mL extraction solution. Alumina column B was a 300 × 22 glass column containing 3 cm of alumina (Fisher A-540, 80-200 mesh), prewet with 25 mL DMF.)

HPLC VARIABLES

Column: 150 × 3.9 5 μm Resolve spherical C18 (Waters)
Mobile phase: MeOH:water 35:65
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: UV 305

CHROMATOGRAM

Retention time: 4.7
Limit of quantitation: 10 ppb

OTHER SUBSTANCES

Simultaneous: furazolidone, nitrofurazone
Noninterfering: pyrantel

KEY WORDS

protect from light; SPE

REFERENCE

Roybal,J.E.; Munns,R.K.; Shimoda,W. Liquid chromatographic determination of carbadox residues in animal feed, *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 653–657.

SAMPLE

Matrix: feed

Sample preparation: Blend 10-100 g feed with 200 mL chloroform:MeOH 75:25 for 3 min, filter through 25 mm Celite 545, re-extract residue with 200 mL chloroform:MeOH 75:25, add 50 mL chloroform:MeOH 75:25 to the residue, filter this mixture, wash the filter cake with several small portions of chloroform:MeOH 75:25. Evaporate the filtrate on a steam bath with a current of air to about 100 mL. Shake the organic layer with 50 mL 10% NaCl in 100 mM NaOH for 1 min, wash the aqueous phase with two or three 50 mL portions of chloroform (until wash is colorless), add 10 mL 1 M KH_2PO_4 solution to the aqueous phase, extract with three 50 mL portions of chloroform. Combine the extracts and filter them through a 25 mm layer of sodium sulfate, evaporate most of the filtrate on a steam bath with a current of air, evaporate the remainder with a current of air. Take

up the residue in 100 mL MeOH, remove a 20 mL aliquot, evaporate most on a steam bath with a current of air, evaporate the remainder with a current of air, dissolve the residue in 100 μL MeOH, make up to 10 mL with MeCN:water 25:75, filter (0.45μm), inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:buffer 25:75 (Buffer was 0.5% acetic acid and 0.05% sodium 1-octanesulfonate.)
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 4.5

OTHER SUBSTANCES
Simultaneous: sulfamethazine

REFERENCE
McGary,E.D. Quantitative determination of sulphamethazine and carbadox in animal feeds by paired ion high-performance liquid, *Analyst*, **1986**, *111*, 1341–1342.

SAMPLE
Matrix: feed
Sample preparation: Grind feed to pass 20-mesh sieve. Add 10 g feed to 10 mL water with swirling, let stand for 5 min (make sure all particles are wet), add 50 mL DMF: water 95:5, shake vigorously for 15 s, shake on a wrist action shaker for 30 min, filter (paper), pass 15 mL through 8 g alumina in a 300 × 10 glass column, if necessary dilute eluate with DMF:water 95:5, inject a 20 μL aliquot. (The alumina was Alcoa F-20, 80-200 mesh, Sargeant-Welch No. SC 10492-005LB, do not substitute.)

HPLC VARIABLES
Guard column: 100 × 2 μBondapak C18/Corasil
Column: Partisil 10 ODS-3
Mobile phase: DMF:buffer 23.5:76.5 (22.3 g $Na_4P_2O_7.H_2O$ and 50 mL phosphoric acid in 700 mL water, add 235 mL DMF, adjust pH to 1.9 ± 0.1 with phosphoric acid , make up to 1 L. Shake DMF with activated carbon and filter before use.)
Flow rate: 1.5
Injection volume: 20
Detector: UV 313

CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Simultaneous: morantel, pyrantel
Noninterfering: lincomycin, tylosin, furazolidone, nitrofurazone, ethopabate, amprolium, sulfa drugs, chlortetracycline, oxytetracycline

REFERENCE
Thorpe,V.A. A collaborative study: high-pressure liquid chromatographic determination of carbadox and pyrantel tartrate in animal feeds, *J.Chromatogr.Sci.*, **1988**, *26*, 545–550.

SAMPLE
Matrix: feed

Sample preparation: Grind and sieve feed with a Moulinex blender. Weigh out 10 g and add it to 20 mL DMF and 60 mL carbon tetrachloride, stir magnetically at 500 rpm at 60° for 30 min, cool, filter (100 μm glass), wash the residue with a little carbon tetrachloride. Remove 25 mL of the filtrate and add it to 45 mL water, stir vigorously for 2 min, centrifuge at 320 g for 5 min, inject an aliquot of the aqueous layer.

HPLC VARIABLES

Column: 250 × 4.1 10 μm Versapack C18 (Alltech)
Mobile phase: Gradient. MeOH:water 15:85 for 4 min, to 50:50 over 2 min, maintain at 50:50 for 4 min, return to initial conditions over 2 min.
Flow rate: 1.5
Injection volume: 20
Detector: UV 305

CHROMATOGRAM

Retention time: 8.5

OTHER SUBSTANCES

Extracted: olaquindox (UV 262 nm)

KEY WORDS

protect from light

REFERENCE

dos Ramos,F.J.; da Silveira,I.N.; de Graaf,G. Column liquid chromatographic determination of carbadox and olaquindox in feeds, *J.Chromatogr.*, **1991**, *558*, 125–130.

SAMPLE

Matrix: solutions
Sample preparation: Dissolve in chloroform at a concentration of 1 μg/mL, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4 5 μm Lichrospher RP-18
Mobile phase: MeCN:10 mM sodium acetate 20:80, pH 5
Column temperature: 30
Flow rate: 1.6
Injection volume: 20
Detector: UV 365

CHROMATOGRAM

Retention time: 5.9
Limit of detection: 11 ng/mL

OTHER SUBSTANCES

Simultaneous: degradation products, nitrofurazone, nitrofurantoin, furazolidone, furaltadone

REFERENCE

Kaniou,I.; Zachariadis,G.; Kalligas,G.; Tsoukali,H.; Stratis,J. Separation and determination of carbadox, nitrofurazone, nitrofurantoin, furazolidone, and furaltadone in their mixtures by thin layer and high performance liquid chromatography, *J.Liq.Chromatogr.*, **1994**, *17*, 1385–1398.

SAMPLE

Matrix: tissue
Sample preparation: Homogenize (Waring blender) 10 g muscle, liver, or kidney in 100 mL EtOH for 5 min, let stand for 5 min, filter through 10 g Celite 545 on top of a sintered

glass filter, rinse blender with 100 mL EtOH and filter rinse. Add 25 mL 3.6% aqueous metaphosphoric acid to the combined filtrates, evaporate to 25 mL under reduced pressure at 45°. Remove residue, rinse out flask with 5 mL hexane and 3 mL water, combine, centrifuge at 0° at 27000 g for 30 min, discard hexane, rinse surface with 5 mL hexane, discard hexane. Remove aqueous layer, rinse out tube twice with 3 mL portions of water, combine, add 10 mL 1 M KH_2PO_4, make up to 100 mL with water, extract three times for 5 min with 50 mL ethyl acetate. Combine the extracts and dry them over 15 g anhydrous sodium sulfate, filter through glass wool, evaporate to dryness under reduced pressure at 45°. Take up residue in 3 mL ethyl acetate and add to alumina column, rinse flask with 2 mL ethyl acetate and add rinse to column. Elute with 20 mL EtOH:MeOH: ethyl acetate 10:10:80 and combine all the eluate. Evaporate to dryness under reduced pressure at 45°, reconstitute in 500 μL mobile phase, inject a 100 μL aliquot. (Prepare alumina column by slurrying 1 g aluminum oxide (Baker) in 20 mL ethyl acetate and adding to a 200 × 6 glass chromatographic column.)

HPLC VARIABLES

Guard column: Brownlee 10 μm RP-GU MPLC C-8
Column: 250 × 4.6 Brownlee RP-10A C-8
Mobile phase: MeCN:EtOH:10 mM ammonium acetate 25:5:70, pH 6.8
Flow rate: 1
Injection volume: 100
Detector: UV 350

CHROMATOGRAM

Retention time: 5.0
Limit of detection: 2 ng
Limit of quantitation: 10 ng

OTHER SUBSTANCES

Extracted: quinoxaline-2-carboxylic acid, furazolidone, nitrofurazone, desoxycarbadox

KEY WORDS

protect from light; pig; muscle; liver; kidney

REFERENCE

MacIntosh,A.I.; Neville,G.A. Liquid chromatographic determination of carbadox, desoxycarbadox, and nitrofurazones in pork tissues, *J.Assoc.Off.Anal.Chem.*, **1984**, *67*, 958–962.

SAMPLE

Matrix: tissue
Sample preparation: Blend (stomacher) 10 g tissue with 40 mL MeCN:MeOH for 3 min, centrifuge at 2000 g for 5 min, add the supernatant to a 400 × 10 column having 2 g 75-150 μm Florisil on top of 8 g alumina (Woelm neutral, activity 1). Collect the first 10 mL of eluate and evaporate it to 1-1.5 mL under a stream of nitrogen at 40-50°, make up to 4 mL with water, mix, add 2 mL isooctane, extract, centrifuge at 2000 g for 5 min, inject a 2 mL aliquot of the aqueous phase onto column A and elute to waste with mobile phase A, after 20 min backflush the contents of column A onto column B, after 5 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES

Column: A 60 × 4.6 37-50 μm Bondapak C18/Corasil; B 10 × 2.1 37-50 μm Bondapak C18/Corasil + 200 × 3 5 μm ChromSpher C18 (Chrompack)
Mobile phase: A Water; B MeCN:10 mM pH 6 sodium acetate buffer 15:85
Flow rate: A 0.5; B 0.6
Injection volume: 2000

Detector: UV 420 following post-column reaction. The column effluent mixed with 500 mM NaOH pumped at 0.23 mL/min and the mixture flowed through a 2 m × 0.5 mm ID knitted PTFE coil to the detector.

CHROMATOGRAM

Retention time: 6
Limit of detection: 0.5-1 ng/g

OTHER SUBSTANCES

Extracted: metabolites
Simultaneous: furaltadone, furazolidone, nitrofurantoin, nitrofurazone
Noninterfering: chloramphenicol, chlortetracycline, clopidol, dapsone, decoquinate, dimetridazole, dinitolmide, doxycycline, ethopabate, fenbendazole, furnicozone, halofuginone, ipronidazole, methylbenzoquate, nicarbazin, nifursol, nitrovin, olaquindox, oxytetracycline, pyrantel, robenidine, ronidazole, sulfadiazine, sulfadimethoxine, sulfadoxine, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfaquinoxaline, tetracycline, thiophanate, trimethoprim

KEY WORDS

post-column reaction; column-switching; protect from light; muscle; liver; kidney; pig; SPE

REFERENCE

Aerts,M.M.L.; Beek,W.M.J.; Keukens,H.J.; Brinkman,U.A.T. Determination of residues of carbadox and some of its metabolites in swine tissues by high-performance liquid chromatography using on-line pre-column enrichment and post-column derivatization with UV-VIS detection, *J.Chromatogr.*, **1988**, *456*, 105–119.